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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/53, C07K 16/00

A1

(11) International Publication Number: WO 98/37415

(43) International Publication Date: 27 August 1998 (27.08.98)

(21) International Application Number: PCT/US97/13059

(22) International Filing Date: 6 August 1997 (06.08.97)

(30) Priority Data:
08/804,106 20 February 1997 (20.02.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: ULCERATIVE COLITIS pANCA SECRETORY VESICLE ANTIGEN AND METHODS OF USING SAME

(57) Abstract

The present invention provides a substantially pure UC pANCA secretory vesicle antigen, including a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane. Pharmaceutical compositions containing the substantially pure UC pANCA secretory vesicle antigen and a pharmaceutical carrier also are provided. The invention further provides a tolerogenic composition containing the substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, combined with a tolerogizing molecule. In addition, there are provided methods of diagnosing ulcerative colitis in a patient and methods of determining susceptibility to ulcerative colitis in an individual using the substantially pure UC pANCA secretory vesicle antigen. The invention further provides methods of inducing tolerance in a pANCA-positive patient with UC and methods of preventing ulcerative colitis in an individual by administering the substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof.

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ULCERATIVE COLITIS pANCA SECRETORY VESICLE ANTIGEN AND METHODS OF USING SAME

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The invention relates generally to the fields of autoimmunity and inflammatory bowel disease and more specifically to the diagnosis and treatment of ulcerative colitis.

BACKGROUND INFORMATION

Inflammatory bowel disease (IBD) is the collective term used to describe two gastrointestinal disorders of unknown etiology: Crohn's disease (CD) and ulcerative colitis (UC). The course and prognosis of ulcerative colitis, which occurs worldwide and is reported to afflict as many as two million people, varies widely. Onset of ulcerative colitis is predominantly in young adulthood with diarrhea, abdominal pain, and fever the three most common presenting symptoms. The diarrhea may range from mild to severe and often is accompanied by bleeding. Anemia and weight loss are additional common signs of ulcerative colitis. Ten percent to fifteen percent of all patients with inflammatory bowel diseases such as ulcerative colitis will require surgery over a ten year period. In addition, patients with ulcerative colitis are at increased risk for the development of intestinal cancer. Reports of an increasing occurrence of psychological problems, including anxiety and depression, are perhaps not surprising symptoms of what is often a debilitating disease that strikes people in the prime of life.

Unfortunately, the available therapies for ulcerative colitis are few, and both diagnosis and treatment have been hampered by a lack of knowledge regarding the etiology of the disease. What is clear, however, is that the pathogenesis of ulcerative colitis involves immune-mediated damage to the intestinal mucosa. Autoantibodies, specifically antibodies against cytoplasmic components of neutrophils (perinuclear anti-neutrophil cytoplasmic antibodies), have been reported in 68-80% of patients with ulcerative colitis, further supporting a role for immune dysregulation in this disease. However, ulcerative colitis target antigens, which would be useful in diagnosing and treating the large population of ulcerative colitis patients that have pANCA autoantibodies, remain to be identified.

Thus, there is a need for identifying an ulcerative colitis pANCA target antigen which can be used in accurate and reproducible methods of diagnosis and in methods of treating ulcerative colitis. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a substantially pure ulcerative colitis (UC) pANCA secretory vesicle antigen, including a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane. The invention provides a pharmaceutical composition containing a substantially pure UC pANCA secretory vesicle antigen and a pharmaceutical carrier. The invention further provides a tolerogenic composition containing a substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, combined with a tolerogizing molecule.

In addition, the invention provides a method of diagnosing ulcerative colitis in a patient by obtaining a sample from the patient; contacting the sample with substantially pure UC pANCA secretory vesicle antigen, or pANCA-reactive fragment thereof, under conditions suitable to form a complex of the antigen, or pANCA-reactive fragment thereof, and antibody to the antigen; and detecting the presence or absence of the complex, where the presence of the complex indicates that the patient has ulcerative colitis.

The invention further provides a method of determining susceptibility to ulcerative colitis in an individual by obtaining a sample from the individual; contacting the sample with substantially pure UC pANCA secretory vesicle antigen, or pANCA-reactive fragment thereof, under conditions suitable to form a complex of the antigen, or pANCA-reactive fragment thereof, and antibody to the antigen; and detecting the presence or absence of the complex, where the presence of the complex indicates that the individual has increased susceptibility to ulcerative colitis.

The invention also provides a method of inducing tolerance in a pANCA-positive patient with ulcerative colitis by administering to the patient an effective dose of a substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof. If desired, a UC pANCA secretory vesicle antigen or tolerogenic fragment thereof can be combined with a tolerogizing molecule. In addition, the invention provides a method of preventing ulcerative colitis in an individual by administering to the individual an effective dose of a substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof. If desired, a UC pANCA secretory vesicle antigen or

tolerogenic fragment thereof can be combined with a tolerogizing molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1 shows immunohistochemical analysis of human colon and human spleen with biotin-conjugated NANUC-1 or NANUC-2. A. human spleen sample reacted with negative control anti-tetanus toxoid antibody. B. human spleen reacted with NANUC-2. C. human colon reacted with negative control anti-tetanus toxoid antibody. D. human colon reacted with NANUC-1.

Figure 2 shows immunohistochemical analysis of retinal tissue with biotin-conjugated NANUC-1 and NANUC-2. A. human spleen sample reacted with negative control anti-tetanus toxoid antibody. B. human spleen reacted with NANUC-2. C. human retina reacted with negative control anti-tetanus toxoid antibody. D. human retina reacted with NANUC-1.

Figure 3 shows immunohistochemical analysis of pancreatic tissue with biotin-conjugated NANUC-1 and NANUC-2. A. human pancreas reacted with negative control anti-tetanus toxoid antibody. B. human pancreas reacted with NANUC-1. C. human pancreas reacted with NANUC-2.

Figure 4 immunohistochemical analysis of human colonic specimens stained for NANUC-2 reactivity and mast cell markers. A. human colon reacted with goat-anti mast cell tryptase. B. human colon reacted with NANUC-2. C. human colon doubly stained with goat anti-mast cell tryptase and NANUC-2.

Figure 5 shows Western analysis of retinal extracts with NANUC-2. Lane 1: whole cell retinal extract reacted with NANUC-2. Lane 2: cytoplasmic extract reacted with NANUC-2. Lane 3: nuclear neutrophil extract reacted with NANUC-2. Lane 4: whole cell retinal extract reacted with control anti-tetanus toxoid antibody. Lane 5: cytoplasmic extract reacted with control anti-tetanus toxoid antibody. Lane 6: nuclear neutrophil extract reacted with control anti-tetanus toxoid antibody.

Figure 6 shows the amino acid sequences of the NANUC-1 and NANUC-2 heavy and light chains.

DETAILED DESCRIPTION OF THE INVENTION

Chronic immune-mediated damage to the colonic mucosa, as well as extra-colonic manifestations, implicate an autoimmune mechanism in the pathogenesis of ulcerative colitis (UC), a debilitating chronic inflammatory disease affecting two million people worldwide. Although the etiology of the disease is unknown, 68-80% of ulcerative colitis patients produce antibodies defined by reactivity with a cytoplasmic neutrophil protein having a perinuclear distribution (perinuclear anti-neutrophil cytoplasmic antibodies designated pANCA). These antibodies are rarely present in other forms of inflammatory bowel disease and are a familial trait associated with susceptibility to

ulcerative colitis and with disease-associated MHC haplotypes. Despite knowledge of these marker antibodies, the antigens responsible for the UC pANCA reactivity have long eluded identification. The present invention is directed to the identification of a UC pANCA target antigen, and its characterization as a secretory vesicle associated protein expressed in selected cell types.

Recombinant monoclonal antibodies representative of ulcerative colitis serum pANCA have been isolated from an ulcerative colitis lamina propria lymphocyte phage display immunoglobulin library and designated NANUC-1 and NANUC-2 as described in Eggena et al., J. Immunol. 156:4005 (1996). As described herein, immunohistochemical analysis of human colonic tissue with these representative UC pANCA monoclonal antibodies detected a cytoplasmic antigen which localized to secretory vesicle membrane in mast cells (Figure 1). A UC pANCA secretory vesicle antigen also was expressed in mast cells of the skin, lung, stomach, gall bladder and small intestine. As shown in Figures 2 and 3, respectively, retinal ganglion cells and pancreatic tissue also express a UC pANCA secretory vesicle antigen.

Thus, the present invention provides a substantially pure ulcerative colitis (UC) pANCA secretory vesicle antigen, which has immunoreactivity with NANUC-1 and NANUC-2 and the characteristic of being selectively expressed in secretory vesicle membrane. A UC pANCA secretory vesicle target antigen of the invention is a valuable reagent for diagnosing ulcerative colitis and for determining the susceptibility of an asymptomatic individual for developing ulcerative colitis in the future.

A UC pANCA secretory vesicle antigen described herein also provides a valuable reagent for treating patients with ulcerative colitis, a disease which, although poorly understood, ultimately involves immune-mediated tissue damage. Similar to autoimmune disorders such as diabetes mellitus and multiple sclerosis, ulcerative colitis can represent a process of immune dysfunction directed against intrinsic intestinal mucosa cells. A UC pANCA secretory vesicle antigen of the invention is expressed in colonic mucosal mast cells. An immune response against these UC pANCA secretory vesicle antigen-expressing mast cells can therefore play a role in the etiology of ulcerative colitis by promoting mucosal inflammation. Ulcerative colitis can be treated by reducing the immune response against UC pANCA secretory vesicle antigen expressing mast cells. As described below, the invention provides methods of inducing tolerance using a UC pANCA secretory vesicle antigen of the invention.

Inflammatory bowel disease is classified into the general categories of Crohn's disease and ulcerative colitis. Effective methods for differentially diagnosing these diseases are important for optimal medical and surgical treatment strategies. Crohn's disease, also known as regional enteritis, is a disease of chronic inflammation that can involve any part of the gastrointestinal tract. Commonly the distal portion of the small intestine (ileum) and cecum are affected. In other cases, the disease is confined to the small intestine, colon or anorectal region. Crohn's disease occasionally involves the duodenum and stomach, and more rarely the esophagus and oral cavity.

The variable clinical manifestations of Crohn's disease are, in part, a result of the varying anatomic localization of the disease. The most frequent symptoms

of Crohn's Disease are abdominal pain, diarrhea and recurrent fever. Crohn's Disease is commonly associated with intestinal obstruction or fistula, which is an abnormal passage between diseased loops of bowel, for example. Crohn's disease also may involve complications such as inflammation of the eye, joints and skin; liver disease; kidney stones or amyloidosis. In addition, Crohn's Disease is associated with an increased risk of intestinal cancer.

Several features are characteristic of the pathology of Crohn's disease. The inflammation associated with Crohn's Disease, known as transmural inflammation, involves all layers of the bowel wall. Thickening and edema, for example, typically also appear throughout the bowel wall, with fibrosis also present in long-standing disease. The inflammation characteristic of Crohn's Disease also is discontinuous in that segments of inflamed tissue, known as "skip lesions," are separated by apparently normal intestine. Furthermore, linear ulcerations, edema, and inflammation of the intervening tissue lead to a "cobblestone" appearance of the intestinal mucosa, which is distinctive of Crohn's Disease.

A hallmark of Crohn's disease is the presence of discrete aggregations of inflammatory cells, known as granulomas, which are generally found in the submucosa. About half of Crohn's disease cases display the typical discrete granulomas, while others show a diffuse granulomatous reaction or nonspecific transmural inflammation. As a result, the presence of discrete granulomas is indicative of Crohn's Disease, although the absence granulomas also is consistent with the disease. Thus, transmural or discontinuous inflammation, rather than the presence of granulomas, is a preferred diagnostic indicator of Crohn's disease (Rubin and

Farber, Pathology (Second Edition) Philadelphia: J.B. Lippincott Company (1994)).

Ulcerative colitis (UC) is a disease of the large intestine characterized by chronic diarrhea with cramping abdominal pain, rectal bleeding, and loose discharges of blood, pus and mucus. The manifestations of ulcerative colitis vary widely. A pattern of exacerbations and remissions typifies the clinical course of most ulcerative colitis patients (70%), although continuous symptoms without remission are present in some patients with UC. Local and systemic complications of ulcerative colitis include arthritis, eye inflammation such as uveitis, skin ulcers and liver disease. In addition, ulcerative colitis and especially long-standing, extensive disease is associated with an increased risk of colon carcinoma.

Several pathologic features characterize ulcerative colitis in distinction to other inflammatory bowel diseases. Ulcerative colitis is a diffuse disease that usually extends from the most distal part of the rectum for a variable distance proximally. The term left-sided colitis describes an inflammation that involves the distal portion of the colon, extending as far as the splenic flexure. Sparing of the rectum or involvement of the right side (proximal portion) of the colon alone is unusual in ulcerative colitis. The inflammatory process of ulcerative colitis is limited to the colon and does not involve, for example, the small intestine, stomach or esophagus. In addition, ulcerative colitis is distinguished by a superficial inflammation of the mucosa that generally spares the deeper layers of the bowel wall. Crypt abscesses, in which degenerated intestinal crypts are filled with neutrophils, also are typical of ulcerative colitis (Rubin and Farber, *supra*, 1994).

In comparison with Crohn's disease, which is a patchy disease with frequent sparing of the rectum, ulcerative colitis is characterized by a continuous inflammation of the colon that usually is more severe distally than proximally. The inflammation in ulcerative colitis is superficial in that it is usually limited to the mucosal layer and is characterized by an acute inflammatory infiltrate with neutrophils and crypt abscesses. In contrast, Crohn's disease affects the entire thickness of the bowel wall with granulomas often, although not always, present. Disease that terminates at the ileocecal valve, or in the colon distal to it, is indicative of ulcerative colitis, while involvement of the terminal ileum, a cobblestone-like appearance, discrete ulcers or fistulas suggest Crohn's disease. Characteristics that serve to distinguish Crohn's disease from ulcerative colitis are summarized in Table 1 (Rubin and Farber, *supra*, 1994).

As used herein, the term "ulcerative colitis" is synonymous with "UC" and means a disease having clinical features of left-sided colonic disease accompanied by a characteristic endoscopic or histopathologic feature of ulcerative colitis. Clinical features of left-sided colonic disease, as used herein, are rectal bleeding, urgency and tenesmus. The rectal bleeding may be accompanied by mucus discharge.

Additional clinical features that may be present in ulcerative colitis include treatment with topical therapy and recommended or performed total or near-total colectomy. A characteristic endoscopic feature of ulcerative colitis, which when present with

Table 1		
Feature	Crohn's Disease	Ulcerative Colitis
Macroscopic		
Thickened bowel wall	Typical	Uncommon
Luminal narrowing	Typical	Uncommon
"Skip" lesions	Common	Absent
Right colon predominance	Typical	Absent
Fissures and fistulas	Common	Absent
Circumscribed ulcers	Common	Absent
Confluent linear ulcers	Common	Absent
Pseudopolyps	Absent	Common
Microscopic		
Transmural inflammation	Typical	Uncommon
Submucosal fibrosis	Typical	Absent
Fissures	Typical	Rare
Granulomas	Common	Absent
Crypt abscesses	Uncommon	Typical

clinical features of left-sided colonic disease indicates ulcerative colitis, is inflammation that is more severe distally than proximally or continuous inflammation. Additional typical endoscopic features that may be present in ulcerative colitis include inflammation extending proximally from the rectum or shallow ulcerations or the lack of deep ulcerations. A characteristic histopathologic feature of ulcerative colitis, which when present with clinical features of left-sided colonic disease indicates ulcerative colitis, is homogeneous, continuous, predominantly superficial inflammation or a lack of "focality" within biopsy specimens. Additional typical histopathologic features that may be present in ulcerative colitis include the

presence of crypt abscesses or a lack of granulomas. Characteristic clinical features of left-sided colonic disease and characteristic endoscopic and histopathologic features of ulcerative colitis are summarized in Table 2.

The differential diagnosis of patients with chronic inflammatory bowel disease as having either Crohn's disease or ulcerative colitis is important in describing specific patterns of disease, predicting outcomes based on expected natural histories, and in guiding medical and surgical treatment strategies. Extensive clinical, endoscopic, and histopathological criteria, as discussed above, have been developed to classify patients into one or the other category. As can be readily appreciated, methods of distinguishing inflammatory bowel disease based on clinical, endoscopic and histopathological criteria are invasive and cumbersome. Furthermore, in numerous cases, the existing criteria are inadequate to diagnose the type of

Table 2	
A. Clinical features of left-sided colonic disease	1. Rectal bleeding possibly accompanied by mucus discharge 2. Urgency 3. Tenesmus 4. Treatment with topical therapy 5. Recommended or performed total or near-total colectomy
B. Endoscopic features of UC	6. Inflammation that is more severe distally than proximally 7. Continuous inflammation 8. Inflammation extending proximally from the rectum 9. Shallow ulcerations or lack of deep ulcerations
C. Histopathologic features of UC	10. Homogeneous, continuous, predominantly superficial inflammation 11. Lack of "focality" within biopsy specimens 12. Crypt abscesses 13. Lack of granulomas

inflammatory bowel disease. Simpler and more precise diagnostic methods are therefore desirable.

Ulcerative colitis marker antibodies can be used in diagnosing inflammatory bowel disease. As discussed above, anti-neutrophil cytoplasmic antibodies that produce a perinuclear staining pattern (pANCA) are elevated in 68-80% of ulcerative colitis patients and less frequently in other disorders of the colon such as Crohn's Disease (Saxon et al., J. Allergy Clin. Immunol. 86:202-210 (1990), Duerr et al., Gastroenterol. 100:1590-1996 (1991), and Pool et al., Gut 34:46-60 (1993)).

Perinuclear to nuclear staining or cytoplasmic staining with perinuclear highlighting and DNase I sensitivity of pANCA reactivity are indicative of ulcerative colitis. The substantially pure UC pANCA target antigen can be used in convenient methods of diagnosing ulcerative colitis that are a significant improvement over multi-step processes of detecting pANCA, which involve fixed neutrophil staining, immunofluorescence and determination of DNase I sensitivity.

Previous studies using confocal microscopy and enzymatic studies have revealed that an antigen recognized by UC pANCA is localized to heterochromatin and is associated with the inner nuclear membrane (Billing et al., Amer. J. Pathol. 147:979 (1995) and Vidrich et al., J. Clin. Immunol. 15293 (1995)). Based on immunoreactivity with NANUC-1 and NANUC-2, the neutrophil autoantigen has been identified as histone H1 (Eggena et al., FASEB J. 10: A1079, #463 (1996)). As described herein, a UC pANCA secretory vesicle antigen of the invention is selectively expressed in secretory vesicle membrane and is expressed in particular cell types such as mast, retinal ganglion and pancreatic islet cells but not in neutrophils. A UC pANCA secretory vesicle antigen of the invention therefore is distinct from the putative neutrophil autoantigen, histone H1.

Regulated exocytosis is a controlled process involving secretory vesicle formation and delivery of proteins to a particular target membrane in response to an inducing signal. While not wishing to be bound by the following, the subcellular localization and cell type restricted expression pattern of a UC pANCA secretory vesicle antigen indicate that this protein can play a role in regulated exocytosis. In particular, a UC pANCA secretory vesicle antigen of the invention is a secretory vesicle membrane-associated protein expressed in

restricted cell types including mast cells, retinal ganglion and pancreatic islet cells. In contrast to the continuous transport of membrane elements and endosomal contents that occurs in all cell types, regulated exocytosis plays a role in particular cell types. For example, the release of inflammatory mediators in mast cells; the release of neurotransmitters in neurons; and the release of insulin by pancreatic islet cells are each examples of regulated exocytosis (see Bennett and Scheller, Proc. Natl. Acad. Sci., *USA* 90:2559-2563 (1993); Ferro-Novick and Novick, Annu. Rev. Cell Biol. 9:575-599 (1993); and Lledo et al., Trends in Neurosci. 10:426-432 (1994)).

Studies indicate that the proteins involved in regulated exocytosis have been evolutionarily conserved among yeast and higher eukaryotes. While the mechanism of regulated exocytosis is not clear, these proteins mediate specific recruitment of a vesicle subset by an inducing event and delivery of the secretory vesicle to the proper membrane target. Proteins involved in regulated exocytosis have been identified by genetic and biochemical studies and include VAMP (vesicle-associated membrane protein), which is an 18 kDa protein anchored to the cytoplasmic surface of the vesicle by a carboxyl-terminal transmembrane domain. Members of the Rab protein family, which are 21 kDa GTP-binding proteins of the Ras superfamily, may interact directly or indirectly with VAMP proteins. More than 30 Rab proteins with different patterns of expression have been identified, with each Rab protein specifically localizing to different organelles and transport vesicles (see, for example, von Mollard, Trends in Biochem. Sci. 19:164-168 (1994)). Rab3a is the most abundant member of the family and is expressed in neurons, β-islet and mast cells (Wu et al., Trends in Biochem. Sci. 21:472-477 (1996)). Syntaxin, a 35 kDa protein with a carboxyl-terminal

anchor identified in the nerve terminal, is a candidate vesicle docking protein localized to the target membrane (Bennett, Science 257:255-259 (1992)).The SEC1 protein is a hydrophilic protein required for proper transport from the Golgi to the plasma membrane. Thus, at least four families of molecules (VAMP, Rab, syntaxin and SEC1) may be involved in regulated membrane transport.

In addition, the Arf (ADP ribosylation factor) family, a group of structurally and functionally conserved proteins of approximately 21 kDa, also are implicated in regulated exocytosis. The Arf proteins, which are members of the Ras superfamily of regulatory GTP-binding proteins and are active only in the GTP-bound form, are localized predominately to the cytoplasmic face of vesicle membranes and may play a role in vesicle formation (Boman and Kahn, Trends in Biochem. Sci. 20:147-150 (1995)).

As used herein, the term "UC pANCA secretory vesicle antigen" means a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane.

As used herein, the term "substantially pure," when used in reference to a UC pANCA secretory vesicle antigen of the invention, means that the antigen is in a form that is relatively free from contaminating cellular components such as lipids, nucleic acids, unrelated proteins or other cellular material normally associated with a UC pANCA secretory vesicle antigen. Described in Example IIA are specific examples of a substantially pure UC pANCA secretory vesicle antigen that is free of associated cellular material.

The term "immunoreactive with NANUC-1 and NANUC-2," as used herein, refers to a protein that forms an immune complex with the monoclonal antibody NANUC-1 or that forms an immune complex with the monoclonal antibody NANUC-2. One of skill in the art understands that the term immunoreactive with NANUC-1 and NANUC-2 refers to relatively specific binding as compared to a non-specific level of binding obtained, for example, with a control protein such as albumin. Immunoreactivity with NANUC-1 or NANUC-2 is conveniently determined using, for example, ELISA or Western analysis as described herein (see Example IIA and IV).

NANUC-1 and NANUC-2 are recombinant antibodies which are representative of serum UC pANCA by the criteria of immunofluorescence, confocal microscopy and DNase I sensitivity (see, for example, Eggena et al., *supra*, 1996). The amino acid sequences of these recombinant antibodies are shown in Figure 6, and the GenBank accession numbers for NANUC-1 and NANUC-2 are U50342 through U50345.

NANUC-1 and NANUC-2 can be produced and purified, for example, by nickel-chelate affinity chromatography as described in Example V or using commercially available systems such as the pThioHis vector and ProBond™ nickel-charged agarose resin (Invitrogen, Carlsbad, CA). To produce NANUC-1 antibody, one can express SEQ ID NO: 1, which is the nucleic acid sequence of the NANUC-1 heavy chain, in combination with SEQ ID NO: 3, which is the nucleic acid sequence of the NANUC-1 light chain. To produce NANUC-1 antibody, one also can express a nucleic acid sequence encoding SEQ ID NO: 2, which is the amino acid sequence of the NANUC-1 heavy chain, with a nucleic acid sequence encoding SEQ ID NO: 4, which is the amino acid sequence of the NANUC-1 light chain.

NANUC-2 can be prepared by expression of nucleic acid sequences encoding the light and heavy chains of NANUC-2. To produce NANUC-2 antibody, one can express SEQ ID NO: 5, which is the nucleic acid sequence of the NANUC-2 heavy chain, in combination with SEQ ID NO: 7, which is the nucleic acid sequence of the NANUC-2 light chain. To produce NANUC-2 antibody, one also can express a nucleic acid sequence encoding SEQ ID NO: 6, which is the amino acid sequence of the NANUC-2 heavy chain, with a nucleic acid sequence encoding SEQ ID NO: 8, which is the amino acid sequence of the NANUC-2 light chain.

As used herein, the term "selectively expressed in secretory vesicle membrane," when used in reference to an antigen of the invention, means that expression is predominantly localized to secretory vesicle membrane. The term selectively expressed in secretory vesicle membrane indicates that expression of a UC pANCA secretory vesicle antigen of the invention is generally lower or absent in other subcellular compartments than in secretory vesicle membrane.

As described herein, NANUC-1 and NANUC-2 positive mast cells are present in a variety of human tissues including skin, lung, stomach, gall bladder and small intestine and in the KU-812 human mast cell line (Figures 1 and 4). The ganglion cell layer of the retina also expresses a UC pANCA secretory vesicle antigen by immunohistochemistry with NANUC-1 (Figure 2). A UC pANCA secretory vesicle antigen also was expressed in the pancreatic tissue (Figure 3). In contrast, most other cell types did not express detectable levels of a UC pANCA secretory vesicle antigen of the invention. Mucosal epithelial cells, for example, did not express detectable levels of a UC pANCA secretory vesicle antigen. Tissue neutrophils also do not express a UC

pANCA secretory vesicle antigen of the invention since these cells demonstrated perinuclear reactivity with NANUC-1 and NANUC-2, indicative of the histone H1 antigen but did not show cytoplasmic reactivity associated with secretory vesicle membrane.

The term "selectively expressed in mast cells," when used in reference to an antigen of the invention, means that expression is predominantly localized to mast cells. The term selectively expressed in mast cells indicates that expression of a UC pANCA secretory vesicle antigen of the invention is generally reduced or absent in other cell types such as neuroendocrine cells and tissue neutrophil.

The term "selectively expressed in mast and neuroendocrine cells," when used in reference to an antigen of the invention, means that expression is predominantly localized to mast and neuroendocrine cells. The term selectively expressed in mast and neuroendocrine cells indicates that expression of a UC pANCA secretory vesicle antigen of the invention is generally lower or absent in other cell types such as tissue neutrophil.

As described herein, a UC pANCA secretory vesicle antigen can be prepared by SDS-PAGE electrophoresis of human mast cell or retinal tissue extracts (see Example II). Other methods of preparing a UC pANCA secretory vesicle antigen are well known in the art. For example, a UC secretory vesicle antigen can be prepared by one or more of the following methods: gel filtration, affinity chromatography, ion exchange or reversed phase chromatography. In addition, adsorption on hydroxylapatite, chromatofocusing, isoelectric focusing and sucrose or glycerol density gradients also can be used in substantially purifying a UC pANCA secretory vesicle antigen (see, for example, Chapter 38

of Deutscher, *supra*, 1990, and Chapter 8 of Balch et al., Methods in Enzymology, Vol. 257, Academic Press, Inc., San Diego (1995)).

Preparative gel electrophoresis can be useful in preparing a substantially pure UC pANCA secretory vesicle antigen of the invention. For example, a UC pANCA secretory vesicle antigen can be purified with preparative polyacrylamide gel electrophoresis and subsequent elution of the antigen by diffusion or electroelution (see, for example, Chapter 33 of Deutscher, Methods in Enzymology: Guide to Protein Purification, Vol. 182, Academic Press, Inc., San Diego (1990)). Continuous elution gel electrophoresis using a system such as the Model 491 Prep Cell (BioRad, Hercules, CA) can be used to purify a UC pANCA secretory vesicle antigen of the invention. If desired, such continuous elution gel electrophoresis can be combined with further purification steps such as liquid phase preparative isoelectric focusing using, for example, the Rotofor system (BioRad).

Immunoaffinity chromatography also can be used to substantially purify a UC pANCA secretory vesicle antigen of the invention. For example, immunoprecipitation with protein A-Sepharose beads coupled to NANUC-1 or NANUC-2 can be used to substantially purify a UC pANCA secretory vesicle antigen. Methods of affinity chromatography are well known in the art and are described, for example, in Chapters 29, 30 and 38 of Deutscher, *supra*, 1990, and in Chen et al., J. Immunol. 157:2593-2600 (1996), which describes immunoaffinity purification of a mast cell membrane protein. Moreover, fractions enriched for the antigen during the process of purification can be conveniently identified by immunoreactivity with NANUC-1

or NANUC-2. An assay useful in determining immunoreactivity with NANUC-1 or NANUC-2 can be, for example, an ELISA as described in Example IV.

A UC pANCA secretory vesicle antigen of the invention as described herein is localized to secretory vesicles in particular cell types. A UC pANCA secretory vesicle antigen of the invention can be selectively expressed in mast cells. A UC pANCA secretory vesicle antigen of the invention also can be selectively expressed in mast cell and neuroendocrine cells such as retinal ganglion cells. Thus, a source for purifying a UC pANCA secretory vesicle antigen of the invention can be, for example, a human or murine mast cell line, retinal ganglion cell line or pancreatic islet cell line or a tissue from which these cell types can be isolated. A particularly useful source for purifying a UC pANCA secretory vesicle antigen is a mast cell line such as a colonic mucosal mast cell line and especially a human mast cell line. A UC pANCA secretory vesicle antigen of the invention can be purified, for example, from the KU-812 human mast cell line; the 10P2 transformed murine embryo mast cell line (ATCC CRL-2034); or the MC/9 murine mast cell line (ATCC CRL-8306), which is derived from fetal liver.

The invention further provides a pharmaceutical composition containing a substantially pure UC pANCA secretory vesicle antigen and a pharmaceutical carrier.

Pharmaceutical carriers are well known in the art and include aqueous solutions such as physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters. A pharmaceutical carrier can contain a physiologically acceptable compound that acts, for

example, to stabilize a UC pANCA secretory vesicle antigen or increase absorption of the antigen. One skilled in the art knows that the choice of a pharmaceutical carrier, including a physiologically acceptable compound, depends on factors such as the desired route of administration. Such a physiologically acceptable compound can be, for example, a carbohydrate, such as glucose, sucrose or dextrans; an antioxidant, such as ascorbic acid or glutathione; a chelating agent; a low molecular weight protein; or another stabilizer or excipient. Pharmaceutical carriers, including stabilizers and preservatives, are described, for example, in Martin, Remington's Pharm. Sci., 15th Ed. (Mack Publ. Co., Easton, 1975).

The present invention also provides methods of diagnosing ulcerative colitis in a patient using the newly identified UC pANCA secretory vesicle antigen are provided. These methods of diagnosing ulcerative colitis in a patient involve obtaining a sample from the patient; contacting the sample with substantially pure UC pANCA secretory vesicle antigen, or pANCA-reactive fragment thereof, under conditions suitable to form a complex of the antigen, or pANCA-reactive fragment thereof, and antibody to the antigen; and detecting the presence or absence of the complex, where the presence of the complex indicates that the patient has ulcerative colitis.

As used herein, the term "sample" means any biological fluid or tissue having pANCA such as, for example, whole blood, plasma or other bodily fluid or tissue having pANCA, preferably serum.

As used herein, the term "patient" means any animal capable of producing pANCA, including, for example, a human, non-human primate, rabbit, rat or mouse, and having a symptom of inflammatory bowel disease

or suspected of having inflammatory bowel disease. A sample to be assayed according to the methods of the invention can be obtained from any such patient and especially a human patient.

As used herein, the term "complex" is synonymous with "immune complex" and means an aggregate of two or more molecules that results from relatively specific binding between an antigen, such as a protein or peptide, and an antibody. For example, a complex can be formed by relatively specific binding of an UC pANCA secretory vesicle antigen to an antibody against an UC pANCA secretory vesicle antigen.

As used herein, the term "antibody" means a population of immunoglobulin molecules, which can be polyclonal or monoclonal and of any isotype. As used herein, the term antibody encompasses an immunologically active fragment of an immunoglobulin molecule. Such an immunologically active fragment contains the heavy and light chain variable regions, which make up the portion of the antibody molecule that specifically binds an antigen. For example, an immunologically active fragment of an immunoglobulin molecule known in the art as Fab, Fab' or $F(ab')_2$ is included within the meaning of the term antibody.

As used herein, the term "secondary antibody" means an antibody or combination of antibodies, which binds pANCA of UC. Preferably, a secondary antibody does not compete with a UC pANCA secretory vesicle antigen for binding to pANCA. A secondary antibody can be an anti-pANCA antibody that binds any epitope of pANCA. A particularly useful secondary antibody is an anti-IgG antibody having specificity for the class determining portion of pANCA. A useful secondary antibody is specific for the species of the ANCA to be detected. For

example, if human serum is the sample to be assayed, mouse anti-human IgG can be a useful secondary antibody. A combination of different antibodies, which can be useful in the methods of the invention, also is encompassed within the meaning of the term secondary antibody, provided that at least one antibody of the combination binds pANCA of UC.

As used herein, the term "class determining portion," when used in reference to a secondary antibody, means the heavy chain constant-region sequence of an antibody that determines the isotype, such as IgA, IgD, IgE, IgG or IgM. Thus, a secondary antibody that has specificity for the class determining portion of an IgG molecule, for example, binds IgG in preference to other antibody isotypes.

A secondary antibody useful in the invention can be obtained commercially or by techniques well known in the art. Such an antibody can be a polyclonal or, preferably, monoclonal antibody that binds pANCA. For example, IgG reactive polyclonal antibodies can be prepared using IgG or Fc fragments of IgG as an immunogen to stimulate the production of antibodies in the antisera of an animal such as a rabbit, goat, sheep or rodent, as described in Harlow and Lane, Antibodies: A Laboratory Manual New York: Cold Spring Harbor Laboratory (1988).

A monoclonal antibody also is useful in the practice of the invention. As used herein, a monoclonal antibody refers to a population of antibody molecules that contain only one species of idiotope capable of binding a particular antigen epitope. Methods of producing a monoclonal antibody are well known (see, for example, Harlow and Lane, *supra*, 1988). An immunogen useful in generating a monoclonal antibody that binds pANCA can be, for example, human IgG or a Fc fragment of

human IgG, pANCA or a Fab fragment of pANCA. A hybridoma that produces a useful monoclonal antibody can be identified by screening hybridoma supernatants for the presence of antibodies that bind pANCA (Harlow, *supra*, 1988). For example, hybridoma supernatants can be screened using neutrophil and pANCA-positive sera in a radioimmunoassay or enzyme-linked immunosorbent assay. In addition, a monoclonal antibody useful in the invention can be obtained from a number of commercial sources.

The term "detectable secondary antibody" means a secondary antibody, as defined above, that can be detected or measured by analytical methods. Thus, the term detectable secondary antibody includes an antibody labeled directly or indirectly with a detectable marker that can be detected or measured and used in a convenient assay such as an enzyme-linked immunosorbent assay, radioimmunoassay, radial immunodiffusion assay or Western blotting assay, for example. A detectable secondary antibody can be produced, for example, by labeling with an enzyme, radioisotope, fluorochrome or chemiluminescent marker. In addition, a secondary antibody can be rendered detectable using a biotin-avidin linkage such that a detectable marker is associated with the secondary antibody. Labeling of the secondary antibody, however, should not impair binding of the secondary antibody to pANCA of UC. If desired, a multiple antibody system can be used as the secondary antibody as discussed above. In such a system, at least one of the antibodies is capable of binding pANCA of UC and at least one of the antibodies can be readily detected or measured by analytical methods.

A secondary antibody can be rendered detectable by labeling with an enzyme such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase or

urease, for example. A horseradish-peroxidase detection system can be used, for example, with the chromogenic substrate tetramethylbenzidine (TMB), which yields a soluble product in the presence of hydrogen peroxide that is detectable by measuring absorbance at 450 nm. An alkaline phosphatase detection system can be used with the chromogenic substrate *p*-nitrophenyl phosphate, for example, which yields a soluble product readily detectable by measuring absorbance at 405 nm. Similarly, a β-galactosidase detection system can be used with the chromogenic substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG), which yields a soluble product detectable by measuring absorbance at 410 nm, or a urease detection system can be used with a substrate such as urea-bromocresol purple (Sigma Immunochemicals, St. Louis, MO). A secondary antibody can be linked to an enzyme by methods well known in the art (Harlow and Lane, *supra*, 1988) or can be obtained from a number of commercial sources. For example, goat F(ab')2 anti-human IgG-alkaline phosphatase is a useful detectable secondary antibody that can be purchased from Jackson Immuno-Research (West Grove, PA).

A secondary antibody also can be rendered detectable by labeling with a fluorochrome. Such a fluorochrome emits light of ultraviolet or visible wavelength after excitation by light or another energy source. DAPI, fluorescein, Hoechst 33258, R-phycocyanin, B-phycoerythrin, R-phycoerythrin, rhodamine, Texas red or lissamine, for example, is a fluorochrome that can be linked to a secondary antibody and used to detect the presence or absence of a complex. A particularly useful fluorochrome is fluorescein or rhodamine. Methods of conjugating and using these and other suitable fluorochromes are described, for example, in Van Vunakis and Langone, Methods in Enzymology, Volume 74, Part C (1991). A secondary antibody linked to a fluorochrome

also can be obtained from commercial sources. For example, goat F(ab')2 anti-human IgG-FITC is available from Tago Immunologicals (Burlingame, CA).

A secondary antibody also can be rendered detectable by labeling with a chemiluminescent marker. Such a chemiluminescent secondary antibody is convenient for sensitive, non-radioactive detection of pANCA and can be obtained commercially from various sources such as Amersham Lifesciences, Inc. (Arlington Heights, IL).

A secondary antibody further can be rendered detectable by labeling with a radioisotope. An iodine-125 labeled secondary antibody is a particularly useful detectable secondary antibody (see, for example, Harlow and Lane, *supra*, 1988).

A signal from a detectable secondary antibody can be analyzed, for example, using a spectrophotometer to detect absorbance from a chromogenic substrate; a fluorometer to detect fluorescence in the presence of light of a certain wavelength; or a radiation counter to detect radiation, such as a gamma counter for detection of iodine-125. For detection of an enzyme-linked secondary antibody, for example, a quantitative analysis of the amount of ANCA can be made using a spectrophotometer such as an EMAX Microplate Reader (Molecular Devices, Menlo Park, CA) in accordance with the manufacturer's instructions. If desired, the assays of the invention can be automated or performed robotically, and the signal from multiple samples can be detected simultaneously.

The assays of the present invention can be forward, reverse or simultaneous as described in U.S. Patent No. 4,376,110, issued March 8, 1983, to David et al.. In the forward assay, each reagent is sequentially

contacted with UC pANCA secretory vesicle antigen. If desired, separation of bound from unbound reagent can be performed before the addition of the next reagent. In a reverse assay, all reagents are pre-mixed prior to contacting UC pANCA secretory vesicle antigen. A modified reverse assay is described in U.S. Patent No. 4,778,751 issued October 18, 1988, to El Shami et al. In a simultaneous assay, all reagents are separately but contemporaneously contacted with UC pANCA secretory vesicle antigen. As used herein, reagent means any component useful to perform the assays of the present invention, for example, the sample, UC pANCA secretory vesicle antigen, detectable secondary antibody, washing buffer or other solutions.

Separation steps for the various assay formats described herein, including the removal of unbound secondary antibody from the complex, can be performed by methods known in the art (Harlow and Lane, *supra*, 1988). For example, a sample can be washed with a suitable buffer and subsequently separated by filtration, aspiration or magnetic separation methods. A UC pANCA secretory vesicle antigen or a pANCA-reactive fragment thereof can be immobilized on a particulate support, such as on microparticles and the particulate material can be centrifuged, if desired, followed by removal of wash liquid. A UC pANCA secretory vesicle antigen or a pANCA-reactive fragment thereof also can be immobilized on a membrane, filter or well, and a vacuum or liquid absorbing apparatus can by applied to the opposite side of the membrane, filter or well to draw the wash liquid away from the complex.

The term "pANCA-reactive fragment of a UC pANCA secretory vesicle antigen," or "pANCA-reactive fragment" as used herein, means a peptide or polypeptide having an amino acid sequence of a portion of the amino acid

sequence of a UC pANCA secretory vesicle antigen and pANCA-reactive activity as defined by the ability to form a complex with pANCA. A pANCA-reactive fragment of a UC pANCA secretory vesicle antigen can have from about three amino acids to about 200 amino acids. Preferably, a pANCA-reactive fragment of a UC pANCA secretory vesicle antigen has from about five to about fifty amino acids and most preferably from about eight to about twenty amino acids.

A pANCA-reactive fragment of a UC pANCA secretory vesicle antigen can be identified by the ability to form a complex with pANCA. For example, a pANCA-reactive fragment of a UC pANCA secretory vesicle antigen can be identified by its ability to form a complex with pANCA when contacted with pANCA-positive ulcerative colitis sera. Assays for the formation of an antigen-pANCA complex using pANCA-positive sera are well known in the art. For example, an enzyme-linked immunosorbent assay (ELISA) as described in Saxon et al., *supra, 1990,* is particularly useful in identifying a pANCA-reactive fragment of a UC pANCA secretory vesicle antigen that forms a complex with pANCA.

A pANCA-reactive fragment of UC pANCA secretory vesicle antigen further can be identified by its ability to form a complex with a representative UC pANCA monoclonal antibody, such as NANUC-1 or NANUC-2. The NANUC-1 and NANUC-2 monoclonal antibodies can be produced and pure as described in Eggena et al., *supra,* 1996. Nucleic acid sequences encoding the NANUC-1 heavy and light chains are provided as SEQ ID NO: 1 and SEQ ID NO: 3, respectively, and nucleic acid sequences encoding the NANUC-2 heavy and light chains are provided as SEQ ID NO: 5 and SEQ ID NO: 7, respectively. Assays for determining binding to NANUC-1 and NANUC-2 are described in Example IV and in Eggena et al., *supra,* 1996. An ELISA assay,

for example, is particularly useful in identifying a pANCA-reactive fragment of a UC pANCA secretory vesicle antigen.

The present invention also provides a method of determining susceptibility to ulcerative colitis in an individual by obtaining a sample from the individual; contacting the sample with substantially pure UC pANCA secretory vesicle antigen, or pANCA-reactive fragment thereof, under conditions suitable to form a complex of the antigen, or pANCA-reactive fragment thereof, and antibody to the antigen; and detecting the presence or absence of the complex, where the presence of the complex indicates that the individual has increased susceptibility to ulcerative colitis.

Serum pANCA has been classified as a potential immunogenetic susceptibility marker for ulcerative colitis. Serum pANCA is found only very rarely in healthy adults and children who are unrelated to patients with ulcerative colitis. In contrast, ANCAs have an increased frequency among the clinically healthy relatives of patients with ulcerative colitis as compared with environmental and ethnically matched controls (see Shanahan et al., Gastroenterol. 103:456-461 (1992) and Seibold et al., Gastroenterol. 107:532-536 (1994)). Thus, identification of a UC pANCA secretory vesicle antigen provides a valuable reagent and methods for identifying individuals with increased susceptibility to ulcerative colitis. Methods of determining susceptibility to ulcerative colitis in an individual can be particularly useful in screening individuals at risk for ulcerative colitis, such as first and second degree relatives of patients with ulcerative colitis and can be valuable, for example, in genetic counseling or in prophylactically treating an individual determined to have an increased susceptibility to ulcerative colitis.

The term "individual," as used herein, means any animal capable of producing pANCA, including a human, non-human primate, rabbit, rat or mouse, provided that the animal does not have ulcerative colitis as defined by the clinical, endoscopic and histopathologic parameters described herein. An individual can be, for example, an asymptomatic person who is a first or second degree relative of a patient with ulcerative colitis. A sample to be assayed according to the methods of the invention can be obtained from any such individual.

As used herein, the term "susceptibility to ulcerative colitis," when used in reference to an individual, means an inability to resist ulcerative colitis disease-causing factors. As described herein, increased susceptibility to ulcerative colitis in an individual is indicated by the presence of a complex of UC pANCA secretory vesicle antigen and antibody to UC pANCA secretory vesicle antigen which is present in a sample from the individual. Increased susceptibility to ulcerative colitis is an increased inability to resist ulcerative colitis disease-causing factors, as compared with a normal or control individual. A control individual can be, for example, an individual from whom a sample is obtained that does not form a complex when contacted with UC pANCA secretory vesicle antigen, or pANCA-reactive fragment thereof, and is generally a member of a family in which there is a negligible incidence of ulcerative colitis or other inflammatory bowel disease. Increased susceptibility to ulcerative colitis in an individual does not mean that the individual will necessarily develop ulcerative colitis. However, increased susceptibility to ulcerative colitis in an individual is associated with an increased probability of having ulcerative colitis in the future.

As described herein, a UC pANCA target antigen is a secretory vesicle protein expressed on colonic mucosal mast cells, indicating that pathogenesis of ulcerative colitis can involve an immune response to a UC pANCA secretory vesicle antigen expressing mast cells in colonic mucosa. Identification of a UC pANCA secretory vesicle antigen indicates that ulcerative colitis can be treated by inducing tolerance to a UC pANCA secretory vesicle antigen, thereby reducing the pathogenic immune response. The present invention therefore provides a tolerogenic composition useful in treating ulcerative colitis. The tolerogenic composition contains substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, combined with a tolerogizing molecule. The present invention also provides methods of inducing tolerance in a pANCA-positive patient with ulcerative colitis by administering to the patient an effective dose of a tolerogenic composition of the invention.

The invention also provides methods of preventing ulcerative colitis in an individual by administering an effective dose of UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, to the individual. The methods of the invention are particularly useful for preventing ulcerative colitis in an individual having increased susceptibility to ulcerative colitis. Such methods can be particularly useful for preventing ulcerative colitis when an effective dose of UC pANCA secretory vesicle antigen or tolerogenic fragment thereof is administered to a newborn individual.

A patient with ulcerative colitis is a patient having ulcerative colitis, as defined by the presence of clinical features of left-sided colonic disease accompanied by a characteristic endoscopic or

histopathologic feature of ulcerative colitis as defined herein. The term "pANCA-positive patient with ulcerative colitis" is the same as "pANCA-positive patient with UC" and means a patient with ulcerative colitis who has perinuclear anti-neutrophil cytoplasmic antibodies (pANCA).

A tolerogenic composition of the invention contains UC pANCA secretory vesicle antigen, or fragment thereof, and a tolerogizing molecule. Various molecules are known in the art to cause, promote or enhance tolerance. See, for example, U.S. Patent No. 5,268,454, and citations therein. As used herein, the term "tolerogizing molecule" means a molecule, compound or polymer that causes, promotes or enhances tolerogenic activity when combined with UC pANCA secretory vesicle antigen, or fragment thereof. A tolerogizing molecule can be, for example, conjugated to a UC pANCA secretory vesicle antigen, or fragment thereof. Such tolerogizing molecules include, for example, polyethylene glycol and are well known in the art (see, for example, U.S. Patent No. 5,268,454, *supra*).

As used herein, the term "effective dose" means the amount of a UC pANCA secretory vesicle antigen or tolerogenic fragment thereof useful for inducing tolerance in a pANCA-positive patient with ulcerative colitis. For induction of oral tolerance, for example, multiple smaller oral doses can be administered or a large dose can be administered. Such doses can be extrapolated, for example, from the induction of tolerance in animal models (see, for example, Trentham et al., Science 261:1727-1730 (1993)).

An effective dose of UC pANCA secretory vesicle antigen or tolerogenic fragment thereof for inducing tolerance can be administered by methods well known in

the art. Oral tolerance is well-recognized in the art as a method of treating autoimmune disease (see, for example, Weiner, Hospital Practice, pp. 53-58 (Sept. 15, 1995)). For example, orally administered autoantigens suppress several experimental autoimmune models in a disease- and antigen-specific fashion; the diseases include experimental autoimmune encephalomyelitis, uveitis, and myasthenia, collagen- and adjuvant-induced arthritis, and diabetes in the NOD mouse (see, for example, Weiner et al., Ann. Rev. Immunol. 12:809-837 (1994)). Furthermore, clinical trials of oral tolerance have produced positive results in treating multiple sclerosis, rheumatoid arthritis and uveitis. In addition, parenteral administration of a tolerogenic composition of UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, can be used to induce tolerance. Subcutaneous injection, for example, can be used to deliver a tolerogenic composition of UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, to a pANCA-positive patient with ulcerative colitis (Johnson, Ann. Neurology 36(suppl.):S115-S117 (1994)).

The term "tolerogenic fragment of a UC pANCA secretory vesicle antigen," as used herein, means a peptide or polypeptide having an amino acid sequence of a portion of the amino acid sequence of a UC pANCA secretory vesicle antigen and tolerogenic activity as defined by its ability either alone, or in combination with another molecule, to produce a decreased immunological response. A tolerogenic fragment of a UC pANCA secretory vesicle antigen has from about three amino acids to about 200 amino acids. Preferably, a tolerogenic fragment of a UC pANCA secretory vesicle antigen has from about five to about fifty amino acids and most preferably from about eight to about twenty amino acids.

A tolerogenic fragment of a UC pANCA secretory vesicle antigen can be identified using a variety of assays, including *in vitro* assays such as T-cell proliferation or cytokine secretion assays and *in vivo* assays such as the induction of tolerance in murine models of ulcerative colitis. T-cell proliferation assays, for example, are well recognized in the art as predictive of tolerogenic activity (see, for example, Miyahara et al., Immunol. 86:110-115 (1995) or Lundin et al, J. Exp. Med. 178:187-196 (1993)). A T-cell proliferation assay can be performed by culturing T-cells with irradiated antigen-presenting cells, such as normal spleen cells, in microtiter wells for 3 days with varying concentrations of a fragment of a UC pANCA secretory vesicle antigen to be assayed; adding ^{3}H-thymidine; and measuring incorporation of ^{3}H-thymidine into DNA. In such an assay, a fragment of a UC pANCA secretory vesicle antigen can be tested for activity, for example, at concentrations of 20 μg/ml and 40 μg/ml.

A tolerogenic fragment of a UC pANCA secretory vesicle antigen also can be identified using a T-cell cytokine secretion assay as is well known in the art. For example, T cells can be cultured with irradiated antigen-presenting cells in microtiter wells with varying concentrations of a fragment of a UC pANCA secretory vesicle antigen and, after three days, the culture supernatants can be assayed for IL-2, IL-4 or IFN-γ as described in Czerinsky et al., Immunol. Rev. 119:5-22 (1991).

Primary T-cells for use in a T-cell proliferation assay or cytokine secretion assay, for example, can be isolated from lamina propria or peripheral blood. In addition, a convenient source of T-cells for use in an *in vitro* assay for tolerogenic activity can be a T-cell line established from an

ulcerative colitis patient, murine model of ulcerative colitis or a healthy animal immunized with a UC pANCA secretory vesicle antigen. A preferred source of T-cells for use in identifying a tolerogenic fragment of a UC pANCA secretory vesicle antigen is an ulcerative colitis patient.

A T-cell line can be established from a patient with ulcerative colitis, for example, by culturing T lymphocytes with about 1 μg/ml UC pANCA secretory vesicle antigen, which is prepared, for example, from the human KU-812 mast cell line as described in Example II, in the presence of low concentrations of growth-supporting IL-2 (about 10 μg/ml). A T-cell line can be established by culturing T lymphocytes with antigen-presenting cells and feeding the cells on an alternating four to five day cycle with either IL-2 and UC pANCA secretory vesicle antigen or IL-2 alone as described in Nanda et al., J. Exp. Med. 176:297-302 (1992). A cell line that develops into a reliably proliferating cell line dependent on the presence of UC pANCA secretory vesicle antigen is particularly useful in identifying tolerogenic fragments of UC pANCA secretory vesicle antigen. The establishment of T-cell lines from small intestinal mucosa is described, for example, in Lundin et al., *supra*, 1993.

A tolerogenic fragment of UC pANCA secretory vesicle antigen can also be identified by its ability to induce tolerance *in vivo*, as indicated by a decreased immunological response, which can be a decreased T-cell response, such as a decreased proliferative response or cytokine secretion response as described above, or a decreased anti-UC pANCA secretory vesicle antigen antibody titer. A neonatal or adult mouse can be tolerized with a fragment of UC pANCA secretory vesicle antigen, and a T-cell response or anti-UC pANCA secretory vesicle antigen antibody titer can be assayed after

challenging by immunization. For example, a neonatal mouse can be tolerized within 48 hours of birth by intraperitoneal administration of about 100 μg of a fragment of UC pANCA secretory vesicle antigen emulsified with incomplete Freund's adjuvant and subsequently immunized with UC pANCA secretory vesicle antigen at about 8 weeks of age (see, for example, Miyahara, *supra*, 1995). An adult mouse can be tolerized intravenously with about 0.33 mg of a fragment of UC pANCA secretory vesicle antigen, administered daily for three days (total dose 1 mg), and immunized one week later with UC pANCA secretory vesicle antigen. A decreased T-cell response, such as decreased proliferation or cytokine secretion, which indicates tolerogenic activity, can be measured using T-cells harvested 10 days after immunization. In addition, a decreased anti-UC pANCA secretory vesicle antigen antibody titer, which also indicates tolerogenic activity, can be assayed using blood harvested 4-8 weeks after immunization. Methods for assaying a T-cell response or anti-UC pANCA secretory vesicle antigen antibody titer are described above and are well known in the art.

A tolerogenic fragment of UC pANCA secretory vesicle antigen also can be identified using a murine model of ulcerative colitis. Neonatal or adult mice having ulcerative colitis-like disease can be tolerized with a fragment of UC pANCA secretory vesicle antigen as described above, and the T-cell response or anti-UC pANCA secretory vesicle antigen antibody titer assayed. A decreased T-cell response or decreased anti-UC pANCA secretory vesicle antigen antibody titer indicates a decreased immunological response and, thus, serves to identify a tolerogenic fragment of UC pANCA secretory vesicle antigen. In addition, a tolerogenic fragment of UC pANCA secretory vesicle antigen can be identified by the ability to reduce the frequency, time of onset or

severity of colitis in a murine model of ulcerative colitis.

Several well-accepted murine models of ulcerative colitis are useful in identifying a tolerogenic fragment of UC pANCA secretory vesicle antigen. For example, mice deficient in IL-2 as described in Sadlack et al., Cell 75:253-261 (1993), and mice deficient in IL-10 as described in Kühn et al., Cell 75:263-274 (1993) have ulcerative-colitis like disease and are useful in identifying a tolerogenic fragment of UC pANCA secretory vesicle antigen. Furthermore, mice with mutations in T cell receptor (TCR) α, TCR β, TCR β x δ, or the class II major histocompatiblility complex (MHC) as described in Mombaerts et al., Cell 75:275-282 (1993) develop inflammatory bowel disease that resembles ulcerative colitis and, thus, are useful in identifying a tolerogenic fragment of UC pANCA secretory vesicle antigen. Similarly, a fragment of UC pANCA secretory vesicle antigen can be assayed for tolerogenic activity in a SCID mouse reconstituted with CD45RB CD4+ T-cells, which is a well-accepted model of human ulcerative colitis, as described in Powrie et al., Immunity 1:553-562 (1994). Thus, a tolerogenic fragment of UC pANCA secretory vesicle antigen readily can be identified by an *in vitro* or *in vivo* assay described herein or by other assays well known in the art.

A pANCA-reactive or tolerogenic fragment of a UC pANCA secretory vesicle antigen can be identified by screening, for example, fragments of UC pANCA secretory vesicle antigen produced by chemical or proteolytic cleavage. A fragment prepared from a UC pANCA secretory vesicle antigen that is purified from a target cell type such as colonic mucosal mast cells can be particularly useful since such a fragment can have a post-translational modification that contributes to

pANCA-reactive activity or tolerogenic activity. Similarly, suitable fragments can be prepared from UC secretory vesicle antigen purified from a cultured mast cells line such as the KU-812 cell line; 10P2 cell line (ATCC CRL-2034); or MC/9 cell line (ATCC CRL-8306). Methods for chemical and proteolytic cleavage and for purification of the resultant protein fragments are well known in the art (see, for example, Deutscher, Methods in Enzymology, Vol. 182, "Guide to Protein Purification," San Diego: Academic Press, Inc. (1990)). For example, a chemical such as cyanogen bromide or a protease such as trypsin, chymotrypsin, V8 protease, endoproteinase Lys-C, endoproteinase Arg-C or endoproteinase Asp-N can be used to produce convenient fragments of UC pANCA secretory vesicle antigen that can be screened for pANCA-reactive activity or tolerogenic activity using one of the assays described herein.

A pANCA-reactive or tolerogenic fragment of UC pANCA secretory vesicle antigen also can be identified by screening a large collection, or library, of random peptides or peptides of interest for pANCA-reactive activity or tolerogenic activity. Peptide libraries include, for example, tagged chemical libraries comprising peptides and peptidomimetic molecules. Peptide libraries also comprise those generated by phage display technology. Phage display technology includes the expression of peptide molecules on the surface of phage as well as other methodologies by which a protein ligand is or can be associated with the nucleic acid which encodes it. Methods for production of phage display libraries, including vectors and methods of diversifying the population of peptides which are expressed, are well known in the art (see, for example, Smith and Scott, Methods Enzymol. 217:228-257 (1993); Scott and Smith, Science 249:386-390 (1990); and Huse, WO 91/07141 and WO 91/07149). These or other well known

methods can be used to produce a phage display library which can be screened, for example, with one of the disclosed assays for pANCA-reactive activity or tolerogenic activity. If desired, a population of peptides can be assayed for activity *en masse*. For example, to identify a pANCA-reactive fragment of a UC pANCA secretory vesicle antigen, a population of peptides can be assayed for the ability to form a complex with NANUC-2; the active population can be subdivided and the assay repeated in order to isolate a pANCA-reactive fragment of a UC pANCA secretory vesicle antigen from the population.

In addition, a peptide library can be a panel of peptides spanning the entire sequence of a UC pANCA secretory vesicle antigen. For example, a panel of about 75 individual 15-mer peptides spanning the sequence of a UC pANCA secretory vesicle antigen can be synthesized, each overlapping by three residue shifts using the Mimotope cleavable pin technology (Cambridge Research Biochemicals, Wilmington, DE), as described by Geysen et al., Science 235:1184 (1987). Such a panel of peptides can be screened for pANCA-reactive activity or tolerogenic activity using one of the assays described above (see, for example, Miyahara et al., *supra*, 1995).

A library of peptides to be screened also can be made up of peptides of interest, such as a population of peptides having sequences similar to the amino acid sequence of a UC pANCA secretory vesicle antigen but having one or more amino acids that differ from a UC pANCA secretory vesicle antigen. For identifying a tolerogenic fragment of UC pANCA secretory vesicle antigen, peptides of interest also can be peptides derived a UC pANCA secretory vesicle antigen that have appropriate HLA-DR binding motifs as described, for example, in Sette et al., J. Immunol. 151:3163-3170

(1993). A particularly useful population of peptides is a population having a HLA-DR2 binding motif (Yang et al., *supra*, 1993). If desired, peptides of interest can be selected for HLA-DR binding activity as described in Sette et al., *supra*, 1993, prior to screening for tolerogenic activity.

As used herein, the term "fragment" means a peptide, polypeptide or compound containing naturally occurring amino acids, non-naturally occurring amino acids or chemically modified amino acids. A pANCA-reactive or tolerogenic fragment of a UC pANCA secretory vesicle antigen also can be a peptide mimetic, which is a non-amino acid chemical structure that mimics the structure of a peptide having an amino acid sequence derived from a UC pANCA secretory vesicle antigen, provided that the peptidomimetic retains pANCA-reactive activity or tolerogenic activity, as defined herein. Such a mimetic generally is characterized as exhibiting similar physical characteristics such as size, charge or hydrophobicity in the same spatial arrangement found in its peptide counterpart. A specific example of a peptide mimetic is a compound in which the amide bond between one or more of the amino acids is replaced, for example, by a carbon-carbon bond or other bond well known in the art (see, for example, Sawyer, Peptide Based Drug Design, ACS, Washington (1995)).

An amino acid can be one of the twenty naturally occurring amino acids, including, unless stated otherwise, L-amino acids and D-amino acids. An amino acid also can be a compound such as a chemically modified amino acid including an amino acid analog, a naturally occurring amino acid that is not usually incorporated into proteins such as norleucine, or a chemically synthesized compound having properties known in the art to be characteristic of an amino acid, provided that the

compound can be substituted within a peptide such that it retains pANCA-reactive activity or tolerogenic activity. Examples of amino acids and amino acids analogs are listed in Gross and Meienhofer, The Peptides: Analysis, Synthesis, Biology, Academic Press, Inc., New York (1983). An amino acid also can be an amino acid mimetic, which is a structure that exhibits substantially the same spatial arrangement of functional groups as an amino acid but does not necessarily have both the α-amino and α-carboxyl groups characteristic of an amino acid.

A pANCA-reactive or tolerogenic fragment of a UC pANCA secretory vesicle antigen useful in the invention can be produced or synthesized using methods well known in the art. Such methods include recombinant DNA methods and chemical synthesis methods for production of a peptide. Recombinant methods of producing a peptide through expression of a nucleic acid sequence encoding the peptide in suitable host cells including eukaryotic and prokaryotic host cells are well known in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed, Vols 1 to 3, Cold Spring Harbor Laboratory Press, New York (1989).

A pANCA-reactive or tolerogenic fragment of a UC pANCA secretory vesicle antigen useful in the invention also can be produced by chemical synthesis, for example, by the solid phase peptide synthesis method of Merrifield et al., J. Am. Chem. Soc. 85:2149 (1964). Standard solution methods well known in the art also can be used to synthesize a pANCA-reactive or tolerogenic fragment of a UC pANCA secretory vesicle antigen useful in the invention (see, for example, Bodanszky, Principles of Peptide Synthesis, Springer-Verlag, Berlin (1984) and Bodanszky, Peptide Chemistry, Springer-Verlag, Berlin (1993)). A newly synthesized peptide can be purified, for example, by high performance liquid chromatography

(HPLC), and can be characterized using, for example, mass spectrometry or amino acid sequence analysis.

It is understood that limited modifications can be made to a UC pANCA secretory vesicle antigen without destroying its biological function. Similarly, limited modifications can be made to a pANCA-reactive fragment of a UC pANCA secretory vesicle antigen or a tolerogenic fragment of a UC pANCA secretory vesicle antigen without destroying its pANCA-reactive activity or tolerogenic activity. A modification of a UC pANCA secretory vesicle antigen that does not destroy pANCA-reactive activity or a modification of a UC pANCA secretory vesicle antigen that does not destroy tolerogenic activity is within the definition of a UC pANCA secretory vesicle antigen. Similarly, a modification of a pANCA-reactive fragment of a UC pANCA secretory vesicle antigen that does not destroy its ability to form a complex with pANCA is within the definition of a pANCA-reactive fragment of a UC pANCA secretory vesicle antigen. Furthermore, a modification of a tolerogenic fragment of a UC pANCA secretory vesicle antigen that does not destroy its ability to produce a decreased immunological response is within the definition of a tolerogenic fragment of a UC pANCA secretory vesicle antigen. A modification can be, for example, an addition, deletion, or substitution of amino acid residues; substitution of a compound that mimics amino acid structure or function; or addition of chemical moieties such as amino or acetyl groups. The activity of a modified UC pANCA secretory vesicle antigen or a modified fragment of a UC pANCA secretory vesicle antigen can be assayed, for example, using one of the assays for pANCA-reactive or tolerogenic activity described herein.

A particularly useful modification of a UC pANCA secretory vesicle antigen or a pANCA-reactive or

tolerogenic fragment of a UC pANCA secretory vesicle antigen is a modification that confers, for example, increased stability. Incorporation of one or more D-amino acids is a modification useful in increasing stability of a protein or protein fragment. Similarly, deletion or substitution of lysine can increase stability by protecting against degradation. For example, such a substitution can increase stability and, thus, bioavailability of a UC pANCA secretory vesicle antigen or a tolerogenic fragment of a UC pANCA secretory vesicle antigen, provided that the substitution does not affect tolerogenic activity.

The present invention also provides a method of inducing tolerance in a pANCA-positive patient with UC by removing sera from the patient; contacting the sera with UC pANCA secretory vesicle antigen, or a pANCA-reactive fragment thereof, under conditions suitable to form a complex of UC pANCA secretory vesicle antigen, or pANCA-reactive fragment thereof, and pANCA; separating the complex from sera lacking the complex; and returning the sera lacking the complex to the patient.

Purification of a UC pANCA secretory vesicle antigen, as described herein, permits isolation of the nucleic acid sequence encoding a UC pANCA secretory vesicle antigen. For example, microsequencing of a UC pANCA secretory vesicle antigen or a fragments thereof using a gas phase Porton/Beckman instrument (Beckman, Palo Alto, CA) can be used to determine the amino acid sequence of the antigen. Preparation of degenerate probe encoding a portion of a UC pANCA secretory vesicle antigen can be used to screen a mast cell cDNA library, for example, in order to obtain a nucleic acid sequence encoding a UC pANCA secretory vesicle antigen as described in Innis et al., PCR Protocols: A Guide to Methods and Application, San Diego: Academic Press, Inc.

(1990); see especially Chapters 5 and 6). A nucleic acid sequence encoding a UC pANCA secretory vesicle antigen also can be obtained by screening a mast cell cDNA library, for example, with NANUC-1 or NANUC-2 as described in Chapter 12 of Sambrook, *supra*, 1989.

An immune response to a UC pANCA secretory vesicle antigen expressed in colonic mucosal mast cells can be involved in mucosal inflammation and the pathogenesis of ulcerative colitis. Thus, methods of inhibiting expression of a UC pANCA secretory vesicle antigen in mast cells can be used to reduce the pathogenic immune response and ameliorate ulcerative colitis. Expression of a UC pANCA secretory vesicle antigen can be inhibited, for example, by treating an individual with a nucleic acid sequence encoding a UC pANCA secretory vesicle antigen in the antisense orientation. Alternatively, one can inhibit expression of a UC pANCA secretory vesicle antigen by modulating the expression of one or more transcription factors that regulate expression of a UC pANCA secretory vesicle antigen gene.

The invention further provides a method of determining susceptibility to ulcerative colitis in an individual by detecting a disease-associated allele at a UC pANCA secretory vesicle antigen locus in an individual, where the presence of a disease-associated UC pANCA secretory vesicle antigen allele indicates increased susceptibility to ulcerative colitis. According to the methods of the invention, a disease-associated allele can be detected by obtaining material from an individual; preparing nucleic acid from the material; contacting the nucleic acid with a disease-associated allele-specific oligonucleotide probe under conditions suitable for formation of a specific hybrid between the nucleic acid and the allele-specific

oligonucleotide probe; and assaying for the presence of the specific hybrid, where the presence of the specific hybrid indicates increased susceptibility to ulcerative colitis. The methods of the invention can be particularly useful, for example, in determining susceptibility to UC in a high-risk population, such as first or second degree relatives of patients with ulcerative colitis.

The invention also provides a method of determining susceptibility to ulcerative colitis in an individual by detecting a non-disease-associated allele at a UC pANCA secretory vesicle antigen locus in an individual, where the presence of the non-disease-associated allele indicates decreased susceptibility to ulcerative colitis. Such methods involve obtaining material from an individual; preparing nucleic acid from the material; contacting the nucleic acid with a non-disease-associated allele-specific oligonucleotide probe under conditions suitable for formation of a specific hybrid between the nucleic acid and the allele-specific oligonucleotide probe; and assaying for the presence of the specific hybrid, where the presence of the specific hybrid indicates decreased susceptibility to ulcerative colitis.

As discussed herein above, an individual is an animal capable of producing pANCA, including a human, non-human primate, rabbit, rat or mouse and especially a human, provided that the animal does not have ulcerative colitis. An individual can be, for example, an asymptomatic person who is a first or second degree relative of a patient with ulcerative colitis.

Material is any biological matter from which a nucleic acid can be prepared. For example, material can be whole blood, plasma or other bodily fluid or tissue

that contains nucleic acid. A preferred material is sera, which can be readily obtained by non-invasive means and used to prepare a nucleic acid for the determination of susceptibility to ulcerative colitis according to the methods of the invention.

A locus is a physical location, place or position occupied by a particular gene on a chromosome. A UC pANCA secretory vesicle antigen locus is any nucleic acid or chromosomal segment that encodes a UC pANCA secretory vesicle antigen or that influences expression of a UC pANCA secretory vesicle gene.

An allele is an alternative gene sequence that occupies the same chromosomal locus as another gene sequence. An alternative gene sequence can be any modification or variation of a gene sequence. Alleles at a polymorphic locus can be, for example, alternative gene sequences that differ by a single nucleotide. Alleles at a polymorphic locus which are particularly useful for determining susceptibility to ulcerative colitis in an individual are disease-associated alleles and non-disease-associated alleles.

A nucleic acid is a polynucleotide such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). A nucleic acid can be either single-stranded or double-stranded. To practice the methods of the invention, a particularly useful nucleic acid is genomic DNA, complementary DNA or messenger RNA. One skilled in the art understands that a nucleic acid useful in the invention has at least that region of a UC pANCA secretory vesicle antigen locus encompassing the polymorphic locus defined by a disease-associated allele and an alternative allele or defined by a non-disease-associated allele and an alternative allele.

A UC pANCA secretory vesicle antigen locus disease-associated allele or a disease-associated allele is a particular gene sequence within a UC pANCA secretory vesicle antigen locus that is associated with an increased susceptibility to ulcerative colitis. Such a disease-associated allele can be, for example, a gene sequence resulting in an abnormal coding sequence of a UC pANCA secretory vesicle antigen. As described above, increased susceptibility to ulcerative colitis in an individual refers to an increased probability of having ulcerative colitis in the future. A non-disease-associated allele is a particular gene sequence within a UC pANCA secretory vesicle antigen locus that is associated with a decreased susceptibility to ulcerative colitis.

A disease-associated UC pANCA secretory vesicle antigen allele-specific oligonucleotide probe or allele-specific oligonucleotide probe is a nucleic acid molecule that will form a specific hybrid, under appropriate conditions, with a nucleic acid encompassing a polymorphic locus having the disease-associated UC pANCA secretory vesicle antigen allele, such that the particular disease-associated allele is distinguished from an alternative allele such as an allele that is not associated with the disease. Appropriate conditions for formation of a specific hybrid such that, for example, a single nucleotide mismatch between a nucleic acid and an allele-specific oligonucleotide probe will preclude formation of a hybrid are well known in the art (Sambrook et al., *supra*, 1989).

An allele at a polymorphic locus, such as a disease-associated UC pANCA secretory vesicle antigen allele, can be detected with an allele-specific oligonucleotide probe by a variety of methods including assays using the polymerase chain reaction (PCR). A

disease-associated allele can be detected, for example, using allele-specific oligonucleotide hybridization (see Mullis et al. (ed.), The Polymerase Chain Reaction Boston: Birkhäuser (1994)); denaturing gradient gel electrophoresis (see, for example, Innis et al., *supra*, 1990; or restriction fragment length polymorphisms (Sambrook et al., *supra*, 1989), which are methods well known in the art and encompassed within the invention.

An allele-specific oligonucleotide probe such as a disease-associated UC pANCA secretory vesicle antigen allele-specific oligonucleotide probe preferably is a nucleic acid molecule having from about seven to about thirty-five nucleotides. More preferably, an allele-specific oligonucleotide probe has from about twelve to about thirty-five nucleotides and most preferably has from about seventeen to about twenty-five nucleotides.

Conditions suitable for formation of a specific hybrid are any set of parameters, physical conditions (such as temperature) or chemical conditions (such as pH or salt concentration) such that an oligonucleotide probe will form a hydrogen bonded, sequence-specific association with the nucleic acid target sequence to which the oligonucleotide probe is complementary. Defining such parameters and conditions is routine to one skilled in the art and, for example, is described in Sambrook et al., *supra*, 1989, and Mullis et al., *supra*, 1994.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

UC pANCA secretory vesicle antigen expression

This example demonstrates that representative UC pANCA monoclonal antibodies bind a target antigen localized to secretory vesicle membrane.

A. Immunohistochemistry with NANUC-1 and NANUC-2 reveals a cytoplasmic secretory vesicle antigen

Representative UC pANCA monoclonal antibodies, isolated from a UC lamina propria lymphocyte phage display IgG library and designated NANUC-1 and NANUC-2, were used for immunohistochemistry. A formalin-fixed, paraffin-embedded human colonic specimen was stained by standard immunohistochemistry, using an anti-tetanus toxoid rFab antibody as a negative control (Figure 1, panel C) and using biotin-conjugated NANUC-1 (Figure 1, panel D). NANUC-1 detected a cytoplasmic antigen in the colonic specimen expressed in a small subset of large, granular cells with features indicative of mast cell origin. The subcellular localization of the NANUC-1 reactive antigen was to the membrane of secretory vesicles as could be seen clearly at a higher magnification.

A distinct pattern of NANUC reactivity was present in human spleen (Figure 1, panel B). In contrast to the cytoplasmic, secretory vesicle-associated staining seen in colon, the NANUC reactivity in spleen was nuclear or perinuclear. These results are consistent with expression of the previously identified histone H1 antigen in splenic neutrophil and indicate that a UC pANCA secretory vesicle protein of the invention is not expressed in spleen.

Colon tissue was counterstained for mast cell markers to identify the NANUC-1 reactive cell type. Counterstaining for the mast cell marker tryptase indicated that the NANUC-1 reactivity in colon colocalized with anti-tryptase reactivity to mast cells (Figure 4, panel C). Thus, the UC pANCA secretory vesicle antigen expressing cells in colonic tissue are mast cells.

Immunohistochemistry was performed as follows. Formalin-fixed and paraffin embedded normal human tissues were obtained from the Surgical Pathology Division of the Department of Pathology at UCLA. Antigen retrieval was performed by the use of microwave oven heating in citrate buffer as described in Ian et al., J. Histochem. and Cytochem. 43:97-102 (1995). Recombinant antibodies were prepared as described in Example V (see, also, Eggena et al., *supra*, 1996) and biotin conjugation was performed by established methods as described in Smith and Scott, Methods in Enzymol. 217: 228 (1993). Samples were blocked with 2% bovine serum albumin (BSA) in PBS for 1 hour and stained with a concentration of 0.5-3.0 μg/ml biotin-conjugated NANUC-1 or NANUC-2 in 2% BSA in PBS for about 1 hour. Samples were subsequently incubated with Vectastain peroxidase or alkaline phosphatase (Vector, Burlingame, CA). Reactivity was detected with 3,3'-diaminobenzidine tetrahydrochloride (DAB) or with the Vector CIP substrate kit (Vector).

B. Expression of a UC pANCA secretory vesicle antigen in selected cell types

A comprehensive set of human organs and tissues and murine intestine were formalin-fixed and paraffin-embedded. Immunohistochemistry performed using NANUC-1 and NANUC-2 as described above demonstrated that a UC pANCA secretory vesicle antigen of the invention was present in a variety of human tissues including skin,

lung, stomach, gall bladder and small intestine. A UC pANCA secretory vesicle antigen of the invention also was expressed in the human KU-812 mast cell line (Blom et al., Eur. J. Immunol 22: 2025-2032 (1992)). In addition, murine intestine demonstrated mast cell immunoreactivity with NANUC-1 and NANUC-2, indicating that a UC pANCA secretory antigen has been evolutionarily conserved.

In addition to expression in mast cells, a cytoplasmic UC pANCA secretory vesicle antigen also was present in the ganglion cell layer of the retina as shown in Figure 2 and in pancreatic tissue as shown in Figure 3. In each of these NANUC-1 and NANUC-2 reactive cell types, a UC pANCA antigen was localized to secretory vesicle membrane.

By immunohistochemistry, expression of a UC pANCA secretory vesicle antigen was apparent in mast cells and specialized neuroendocrine cells (Figures 1 to 3) but was not expressed in most other cell types. For example, in the human colonic sample, a UC pANCA secretory vesicle antigen was not expressed in the mucosal epithelial cells. As can be seen in Figure 1B, cells in the spleen specimen expressed the nuclear or perinuclear NANUC reactivity characteristic of histone H1 but did not express a UC pANCA secretory vesicle antigen of the invention. Tissue neutrophils, which also demonstrated perinuclear reactivity with NANUC-1 and NANUC-2, also do not express a UC pANCA secretory vesicle antigen of the invention. These results indicate that a UC pANCA secretory vesicle antigen is selectively expressed in defined cell types.

EXAMPLE II

Isolation of the ulcerative colitis pANCA secretory vesicle antigen

This example describes isolation of a UC pANCA secretory vesicle antigen from a human mast cell line and retinal tissue.

A. Western analysis of a UC pANCA secretory vesicle antigen

Extracts from the KU-812 human mast cell line were analyzed for NANUC-2 reactivity following electrophoresis on 12 % SDS-PAGE under nondenaturing conditions. Western analysis of KU-812 cell extracts demonstrated that proteins of about 80 kDa, about 65 kDa, about 46 kDa, about 40 kDa and about 21 kDa are UC pANCA secretory vesicle antigens. These NANUC-2 immunoreactive proteins were not present in human neutrophil. IN addition, the 80, 65, 46, 40 and 21 kDa proteins did not react with negative control anti-tetanus toxoid antibody, indicating that the immunoreactivity was specific for NANUC-2.

The 80 kDa and 21 kDa UC secretory vesicle antigens were expressed in retinal tissue extracts in addition to mast cells, indicating that UC pANCA secretory vesicle antigens of about 80 kDa and about 21 kDa are selectively expressed in mast and neuroendocrine cells. The 65 kDa, 46 kDa and 40 kDa proteins were expressed in the KU-812 human mast cell line but not in retinal tissue extracts, indicating that UC pANCA secretory vesicle antigens of about 65 kDa, 46 kDa and 40 kDa are selectively expressed in mast cells.

Western analysis of human retinal extracts is shown in Figure 5. As can be seen in the whole cell

retinal extracts, proteins of about 80 kDa and about 21 kDa reacted with NANUC-2 (lane 1). These proteins were not expressed in neutrophil (lane 3) or reactive with negative control anti-tetanus toxoid antibody (lane 4). The cytoplasmic extract sample shown in lane 2 does not contain an 80 or 21 kDa proteins, indicating that secretory vesicles have been lost during preparation of this cytoplasmic extract.

Western analysis was performed as follows using the KU-812 cells obtained from Dr. A. Saxon (Department of Medicine, UCLA) or retinal tissue. Extracts were prepared by medical shearing in HEPES buffer (SIGMA) containing Complete Protease Inhibitors (Boehringer Mannheim, Indianapolis, IN). The cell extracts were electrophoresed on a 12% polyacrylamide gel under nondenaturing conditions. Proteins were transferred to nitrocellulose membranes, and the transfer verified by Ponceau S red staining (SIGMA, St. Louis, MO). Membranes were blocked with 5% milk in 0.1% Tween-20/PBS for 1 hour. Primary and secondary antibody incubations were for 1 hour in 1% milk in 0.1% Tween-20/PBS. The primary antibodies, NANUC-1, NANUC-2, and anti-tetanus toxoid Fabs were used at a concentration of 0.1 to 4.0 μg/ml. The secondary antibody was goat anti-human Fab-alkaline phosphatase or goat anti-human kappa-biotin used at a dilution of 1 to 1000 or 1 to 2000, respectively. Alkaline phosphatase labeled antibodies were detected with BCIP-NBT (SIGMA). Biotinylated antibodies were detected with SA-HRP (Amersham Lifesciences, Inc., Arlington Heights, IL) and enhanced chemiluminescence.

B. Purification of a UC pANCA secretory vesicle antigen

KU-812 cells or human retinal tissue is fractionated according to Tooze and Hutner, Cell 60:837 (1990). Fractions containing UC pANCA secretory vesicle

antigen are isolated by affinity purification as described in Chapter 8.2 of Coligan et al., Current Protocols in Immunology, J. Wylie and Sons (1996). Following purification, protein microsequencing is used to determine the amino acid sequence of the NANUC-1 and NANUC-2 reactive antigen.

EXAMPLE III

Expression of a UC pANCA secretory vesicle antigen in ulcerative colitis, Crohn's Disease and normal colonic tissue

This example demonstrates that a UC pANCA secretory vesicle antigen is expressed at a comparable frequency in normal individuals and those with ulcerative colitis or Crohn's Disease.

Immunohistochemistry with NANUC-1 or NANUC-2 was performed as described in Example I with 33 colonic specimens equally representing patients with ulcerative colitis, Crohn's Disease and normal colon (uninvolved specimens from cancer resections). Tissues were blindly evaluated and positive cells were quantitatively scored for both the mucosal and submucosal compartments.

As described above, the NANUC-1 and NANUC-2 monoclonal antibodies detected a cytoplasmic antigen which localized to secretory vesicles and was expressed in a small subset of large granular cells with features indicating mast cell origin. The frequency of NANUC-positive cells was heterogeneous (0.2 to 17 cells per unit field; mean +/- SEM, 4.4 +/- 0.4). The abundance of NANUC-positive cells in each specimen was generally concordant in mucosal and submucosal layers. Comparison of the number of NANUC-positive cells in samples derived from patients with ulcerative colitis, Crohn's disease or normal colon indicated that expression

of a UC pANCA secretory vesicle antigen in colon was not correlated with disease status.

EXAMPLE IV

ELISA Analysis with NANUC-1 and NANUC-2

This example describes ELISA analysis of UC pANCA secretory vesicle antigen and fragments thereof using NANUC-1 and NANUC-2.

Microtiter plates are coated with neutrophil or with substantially pure UC pANCA secretory vesicle antigen or fragment of interest. The reactivity of NANUC-1, NANUC-2 or negative control anti-tetanus toxoid antibody is tested against antigen in ELISA assays performed as follows. Microtiter plates (Costar 3069, Cambridge, MA) are coated overnight at 4°C with 500ng/well antigen in bicarbonate pH 9.6 coating buffer. Wells are blocked with 0.25% BSA/PBS for 1 hour; incubated with NANUC-1 or NANUC-2 diluted in 0.25% BSA/PBS for 1 hour; and washed five times with 0.5% Tween-20/PBS at room temperature. Plates are subsequently incubated with a 1 to 1000 dilution of alkaline phosphatase-labeled goat anti-human Fab (Pierce, Rockford, IL) for 1 hour; washed five times in 0.5% Tween-20/PBS; washed three times with Tris-NaCl (50 mM Tris/150 mM NaCl pH 7.5); and developed with 5 mg/ml *p*-nitrophenyl phosphate (SIGMA) in 10% diethanolamine/1 mM $MgCl_2$ pH 9.8. The absorbance of each sample is measured at 405 nm using a BioRad ELISA reader (Richmond, CA). Neutrophil samples are prepared as described in Saxon et al., *supra*, 1990, and UC pANCA secretory vesicle antigen is prepared as described in Example II.

EXAMPLE V

Preparation of NANUC-1 and NANUC-2

This example describes preparation of the recombinant UC monoclonal antibodies NANUC-1 and NANUC-2 by nickel-chelate affinity chromatography.

Vector $C_3AP313H_6$ (provided by Dr. Carlos Barabas III, Scripps Research Institute, La Jolla, CA), a pComb3 derivative, was digested with Spe I/Nhe I and engineered to express NANUC-1 heavy and light chains with six histidines at the carboxy-terminal end of the Fab. Vector $C_3AP313H_6$ was digested with Spe I/Nhe I and engineered to express NANUC-2 heavy and light chains with six histidines at the carboxy-terminal end of the Fab.

A 10 ml overnight culture derived from one colony with NANUC-1 vector or NANUC-2 vector was used to inoculate 1 liter of super broth containing 50 μg/ml carbenicillin and 20 mM $MgCl_2$. The culture was incubated at 37° C (300 rpm) until an absorbance (OD_{600}) of 0.6 to 0.8 and then induced with 4 mM IPTG and grown at 30°C for approximately 16 hours. Bacteria were pelleted and resuspended in 30 ml sonication buffer (300 mM NaCl/50 mM $NaPO_4$/0.01% NaN_3, pH 7.9) and sonicated for 8 x 15 second bursts at 50% power using a 40 watt microsonic disrupter (Tekmar, Cincinnati, OH). The sonicate was centrifuged at 15,000 rpm in a Beckman JA-20 (Beckman Instruments, Palo Alto, CA) for 40 minutes at 4° C, and the supernatant serially passed through 0.45 and 0.22 micron filters (Corning, Corning, NY).

Nickel-affinity chromatography was performed as follows. The sonicate was immediately loaded at 20 ml/h on a 1 ml nickel-nitriloacetate column (Qiagen, Chatsworth, CA) and washed with sonication buffer, approximately 40 to 50 ml, until OD_{280} of less than 0.01

was obtained. The column was then washed with 10 ml of 10 mM imidazole in sonication buffer to remove contaminants, followed with 10 ml each of 100 mM, 250 mM, and 500 mM imidazole. Fractions of 1 ml volume were monitored at OD_{280}, and aliquots were analyzed by SDS-PAGE (12%, reducing) to confirm NANUC-1 and NANUC-2 Fab identity and purity. Due to the presence of imidazole, samples were not boiled but instead were denatured at 37° C for 10 minutes before loading. Typically, the Fab eluted in the first three fractions of the 100 mM imidazole wash. Fractions containing the NANUC-1 or NANUC-2 Fab were then pooled and dialyzed (6-8 kDa cutoff membranes (Spectrum Medical Industries, Los Angeles, CA) against PBS.

The NANUC-1 and NANUC-2 preparations were greater than 90% pure for intact Fab as determined by total protein densitometry of reduced and nonreduced samples analyzed with SDS-PAGE and Coomassie blue staining. The calculated Ab levels in the purified fraction were the same for SDS-page densitometry and a colorimetric protein assay (BioRad, Richmond, CA). However, anti-κ ELISA using a polyclonal Fab' standard detected a reduced recombinant Fab concentration: for 1 liter of bacterial culture, the Fab yield was about 1 mg by protein assay versus about 0.1 to 0.3 mg by immunoassay. This difference was attributed to effects of the bacterial expression system on constant region epitope detection.

Although the invention has been described with reference to the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Regents of University of California
Cedars-Sinai Medical Center

(ii) TITLE OF INVENTION: Ulcerative Colitis pANCA Secretory Vesicle Antigen and Methods of Using Same

(iii) NUMBER OF SEQUENCES: 8

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 06-AUG-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/804,106
(B) FILING DATE: 20-FEB-1997

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 699 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..699

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..699
(D) OTHER INFORMATION: /note= "product = NANUC-1 Heavy Chain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GCC CAG GTG AAA CTG CTC GAG CAG TCT GGG GGA GGC GTG GTC CAG CCT      48
Ala Gln Val Lys Leu Leu Glu Gln Ser Gly Gly Gly Val Val Gln Pro
 1               5                  10                  15

GGG AAG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGG      96
Gly Lys Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg
            20                  25                  30

AAC TAT GGC ATG CAC TGG GTC CGG CAG GCT CCA GGC AAG GGG CTG GAG     144
Asn Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
        35                  40                  45

TGG GTG GCA GGT ATT TCC TCT GAT GGA AGA AAA AAA AAG TAT GTA GAC     192
Trp Val Ala Gly Ile Ser Ser Asp Gly Arg Lys Lys Lys Tyr Val Asp
    50                  55                  60

TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAG TCC AAG AAC ACG     240
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Lys Ser Lys Asn Thr
65                  70                  75                  80

CTG TAT CTG CAA ATG AAC AGC CTC AGA GCT GAG GAC ACG GCT GTG TAT     288
Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95

TAC TGT GCG AAA TTG TCC CGC GCG GGT GGT TTT GAC ATC TGG GGC CAA     336
Tyr Cys Ala Lys Leu Ser Arg Ala Gly Gly Phe Asp Ile Trp Gly Gln
            100                 105                 110

GGG ACA ATG GTC ACC GTC TCT TCA GCC TCC ACC AAG GGC CCA TCG GTC     384
Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

TTT CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC     432
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG     480
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC     528
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC     576
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG     624
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

CCC AGC AAC ACC AAG GTG GAC AAG AAA GCA GAG CCC AAA TCT TGT GAC     672
Pro Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp
    210                 215                 220

AAA ACT AGT CAC CAC CAC CAC CAC CAC                                 699
Lys Thr Ser His His His His His His
225                 230
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ala Gln Val Lys Leu Leu Glu Gln Ser Gly Gly Gly Val Val Gln Pro
 1               5                  10                  15

Gly Lys Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg
            20                  25                  30

Asn Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
        35                  40                  45

Trp Val Ala Gly Ile Ser Ser Asp Gly Arg Lys Lys Lys Tyr Val Asp
    50                  55                  60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Lys Ser Lys Asn Thr
65                  70                  75                  80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Cys Ala Lys Leu Ser Arg Ala Gly Gly Phe Asp Ile Trp Gly Gln
            100                 105                 110

Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
        115                 120                 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
    130                 135                 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr Ser His His His His His His
225                 230
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 642 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..642

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..642
(D) OTHER INFORMATION: /note= "product = NANUC-1 light chain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GCC GAG CTC ACG CAG TCT CCA GGC ACC CTG TCT TTG TTT CCA GGG GAA      48
Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Phe Pro Gly Glu
 1               5                  10                  15

AGA GCC ACT CTC TCC TGC AGG GCC AGT CAG AGA ATT AGC ACC AGT TTC      96
Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Ile Ser Thr Ser Phe
            20                  25                  30

TTA GCC TGG TAC CAG CAG AAG CCT GGC CAG TCT CCC AGG CTC CTC ATC     144
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Arg Leu Leu Ile
        35                  40                  45

TTT GAT GCA TCC ACC AGG GCC CCT GGC ATC CCT GAC AGG TTC AGT GCC     192
Phe Asp Ala Ser Thr Arg Ala Pro Gly Ile Pro Asp Arg Phe Ser Ala
    50                  55                  60

AGT TGG TCT GGG ACA GAC TTC ACT CTC ACC ATC AGC AGA CTG GAG CCT     240
Ser Trp Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
65                  70                  75                  80

GAA GAT TTT GCA GTC TAT TAC TGT CAA CAT TAT GGT GGG TCT CCC TGG     288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Gly Ser Pro Trp
                85                  90                  95

ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAG CGA ACT GTG GCT GCA     336
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA     384
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC     432
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG     480
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160
```

```
GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC      528
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC      576
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC      624
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

TTC AAC AGG GGA GAG TGT                                              642
Phe Asn Arg Gly Glu Cys
    210
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 214 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Phe Pro Gly Glu
 1               5                  10                  15

Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Ile Ser Thr Ser Phe
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Arg Leu Leu Ile
        35                  40                  45

Phe Asp Ala Ser Thr Arg Ala Pro Gly Ile Pro Asp Arg Phe Ser Ala
    50                  55                  60

Ser Trp Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
65                  70                  75                  80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Gly Ser Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205
```

```
Phe Asn Arg Gly Glu Cys
    210
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 732 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..732

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..732
(D) OTHER INFORMATION: /note= "product = NANUC-2 Heavy Chain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CTC GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AAG TCC CTG AGA CTC          48
Leu Glu Ser Gly Gly Gly Val Val Gln Pro Gly Lys Ser Leu Arg Leu
  1               5                  10                  15

TCC TGT GCA GCC TCT GGA TTC ACC TTC AGG AAC TAT GGC ATG CAC TGG          96
Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Gly Met His Trp
            20                  25                  30

GTC CGG CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GGT ATT TCC         144
Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Gly Ile Ser
        35                  40                  45

TCT GAT GGA AGA AAA AAA AAG TAT GTA GAC TCC GTG AAG GGC CGA TTC         192
Ser Asp Gly Arg Lys Lys Lys Tyr Val Asp Ser Val Lys Gly Arg Phe
    50                  55                  60

TTC ATC TCC AGA GAC AAT TCC AAG AAC ACC CTG TAT CTG CAA TTG AAC         240
Phe Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Leu Asn
65                  70                  75                  80

AGC CTG AGA GCT GAG GAC ACG GCT GTC TAT TAC TGT GCG AAA GAT GAG         288
Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Asp Glu
                85                  90                  95

TTT AGT TCT ACC CGG AAG AAC TTC TTG ACT GGT CAA TCA AAG ACC TTT         336
Phe Ser Ser Thr Arg Lys Asn Phe Leu Thr Gly Gln Ser Lys Thr Phe
            100                 105                 110

GCG GCC TAC TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC         384
Ala Ala Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr
        115                 120                 125

GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC         432
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
    130                 135                 140

TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC         480
Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
145                 150                 155                 160
```

```
AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC        528
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
                165                 170                 175

CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA        576
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
            180                 185                 190

CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC        624
Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
        195                 200                 205

ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG        672
Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
    210                 215                 220

GTG GAC AAG AAA GCA GAG CCC AAA TCT TGT GAC AAA ACT AGT CAC CAC        720
Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr Ser His His
225                 230                 235                 240

CAC CAC CAC CAC                                                       732
His His His His
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 244 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Leu Glu Ser Gly Gly Gly Val Val Gln Pro Gly Lys Ser Leu Arg Leu
 1               5                  10                  15

Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Gly Met His Trp
            20                  25                  30

Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Gly Ile Ser
        35                  40                  45

Ser Asp Gly Arg Lys Lys Lys Tyr Val Asp Ser Val Lys Gly Arg Phe
    50                  55                  60

Phe Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Leu Asn
65                  70                  75                  80

Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Asp Glu
                85                  90                  95

Phe Ser Ser Thr Arg Lys Asn Phe Leu Thr Gly Gln Ser Lys Thr Phe
            100                 105                 110

Ala Ala Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr
        115                 120                 125

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
    130                 135                 140

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
145                 150                 155                 160
```

```
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
                165                 170                 175

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
            180                 185                 190

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
        195                 200                 205

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
    210                 215                 220

Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr Ser His His
225                 230                 235                 240

His His His His
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 645 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..645

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..645
(D) OTHER INFORMATION: /note= "product = NANUC-2 light chain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
GCC GAG CTC ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA      48
Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu
  1               5                  10                  15

AGA GCC ACC CTC TCC TGC AGG GCC AGT CAG GGT GTT AGC AGC GGC TCC      96
Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Gly Val Ser Ser Gly Ser
            20                  25                  30

TTA GCC TGG TAT CAG CAG AAA GCT GGC CAG GCT CCC AGG CTC CTC ATC     144
Leu Ala Trp Tyr Gln Gln Lys Ala Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

TAT GGT GCA TCC AGG AGG GCC ACT GGC ATC CCA GAC AGG TTC ACT GGC     192
Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Thr Gly
    50                  55                  60

AGT GGG TCT GGG ACA GAC TTC ACT CTC ACC ATC ACC AGA CTG GAG CCT     240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro
65                  70                  75                  80

GAA GAT TTT GCA GTG TAT TAC TGT CAG CAG TAT GGT AGC TCC CAG GGA     288
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Gln Gly
                85                  90                  95
```

```
TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT CTC AAA CGA ACT GTG GCT      336
Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Leu Lys Arg Thr Val Ala
            100                 105                 110

GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT      384
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG      432
Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC      480
Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC      528
Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC      576
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG      624
Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

AGC TTC AAC AGG GGA GAG TGT                                          645
Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 215 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu
 1               5                  10                  15

Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Gly Val Ser Ser Gly Ser
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Ala Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

Tyr Gly Ala Ser Arg Arg Ala Thr Gly Ile Pro Asp Arg Phe Thr Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro
65                  70                  75                  80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Gln Gly
                85                  90                  95

Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Leu Lys Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125
```

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
130 135 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145 150 155 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
165 170 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205

Ser Phe Asn Arg Gly Glu Cys
210 215

We claim:

1. A substantially pure ulcerative colitis (UC) pANCA secretory vesicle antigen, comprising a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane.

2. The substantially pure UC pANCA secretory vesicle antigen of claim 1, comprising a protein selectively expressed in mast cells.

3. The substantially pure UC pANCA secretory vesicle antigen of claim 1, comprising a protein selectively expressed in mast and neuroendocrine cells.

4. A pharmaceutical composition, comprising a substantially pure UC pANCA secretory vesicle antigen and a pharmaceutical carrier, wherein said UC pANCA secretory vesicle antigen is a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane.

5. The pharmaceutical composition of claim 4, wherein said UC pANCA secretory vesicle antigen is a protein selectively expressed in mast cells.

6. The pharmaceutical composition of claim 4, wherein said UC pANCA secretory vesicle antigen is a protein selectively expressed in mast and neuroendocrine cells.

7. A tolerogenic composition, comprising a substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, combined with a tolerogizing molecule, wherein said UC pANCA secretory vesicle antigen comprises a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane.

8. The tolerogenic composition of claim 7, wherein said UC pANCA secretory vesicle antigen is a protein selectively expressed in mast cells.

9. The tolerogenic composition of claim 7, wherein said UC pANCA secretory vesicle antigen is a protein selectively expressed in mast and neuroendocrine cells.

10. A method of diagnosing ulcerative colitis in a patient, comprising the steps of:

(a) obtaining a sample from said patient;

(b) contacting said sample with substantially pure UC pANCA secretory vesicle antigen, or pANCA-reactive fragment thereof, under conditions suitable to form a complex of said antigen, or pANCA-reactive fragment thereof, and antibody to said antigen, wherein said antigen comprises a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane; and

(c) detecting the presence or absence of said complex, wherein the presence of said complex indicates that said patient has ulcerative colitis.

11. The method of claim 10, wherein the presence or absence of said complex is detected with a detectable secondary antibody that has specificity for a class determining portion of said antibody to said antigen.

12. A method of determining susceptibility to ulcerative colitis in an individual, comprising the steps of:

(a) obtaining a sample from said individual;

(b) contacting said sample with substantially pure UC pANCA secretory vesicle antigen, or pANCA-reactive fragment thereof, under conditions suitable to form a complex of said antigen, or pANCA-reactive fragment thereof, and antibody to said antigen, wherein said antigen comprises a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane; and

(c) detecting the presence or absence of said complex, wherein the presence of said complex indicates that said individual has increased susceptibility to UC.

13. The method of claim 12, wherein the presence or absence of said complex is detected with a detectable secondary antibody that has specificity for a class determining portion of said antibody to said antigen.

14. A method of inducing tolerance in a pANCA-positive patient with ulcerative colitis, comprising administering to the pANCA-positive patient with ulcerative colitis an effective dose of a substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, wherein said UC pANCA secretory vesicle antigen comprises a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane.

15. The method of claim 14, wherein said substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, is administered in combination with a tolerogizing molecule.

16. The method of claim 14, wherein a level of autoantibodies reactive with said UC pANCA secretory vesicle antigen in said pANCA-positive patient is reduced.

17. A method of preventing ulcerative colitis in an individual, comprising administering to said individual an effective dose of a substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, wherein said UC pANCA secretory vesicle antigen comprises a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane.

18. The method of claim 17, wherein said substantially pure UC pANCA secretory vesicle antigen, or tolerogenic fragment thereof, is administered in combination with a tolerogizing molecule.

19. The method of claim 17, wherein said individual has increased susceptibility to ulcerative colitis.

20. A composition comprising a substantially pure UC pANCA secretory vesicle antigen in combination with NANUC-1, wherein said UC pANCA secretory vesicle antigen is a protein immunoreactive with NANUC-1 and NANUC-2 and having the characteristic of being selectively expressed in secretory vesicle membrane.

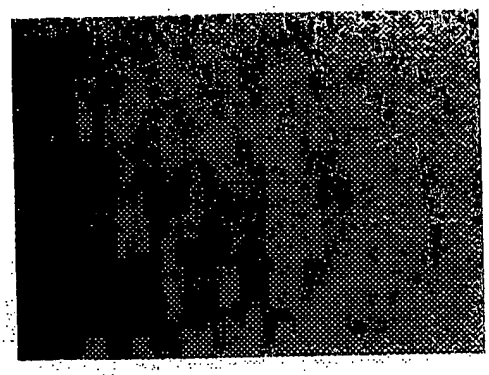

FIG. 1A

FIG. 1B

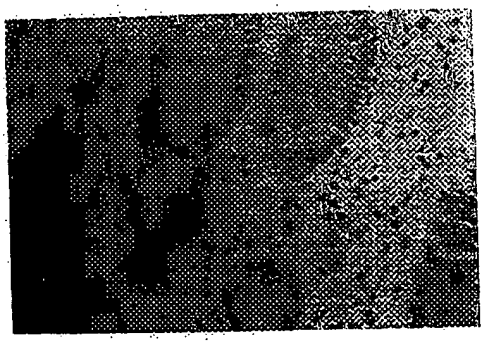

FIG. 1C

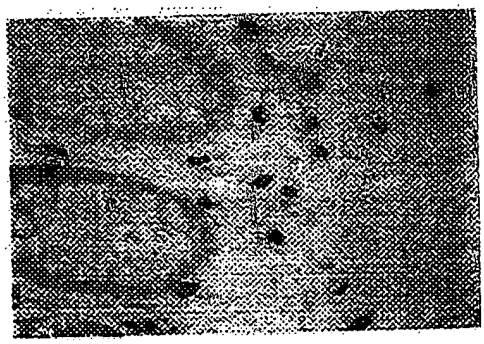

FIG. 1D

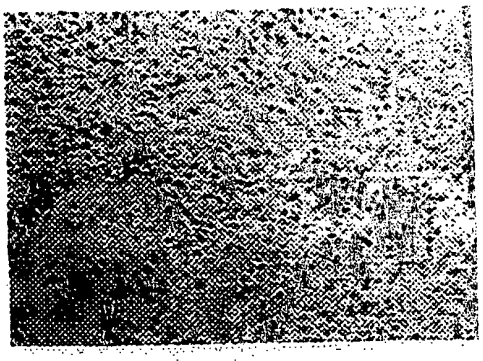

FIG. 2A

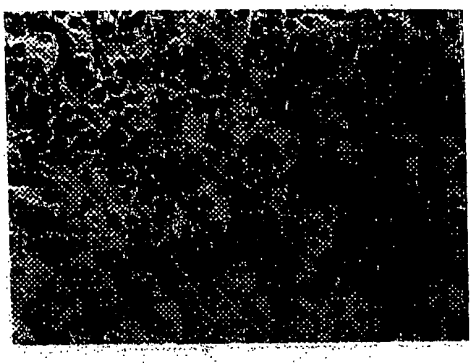

FIG. 2B

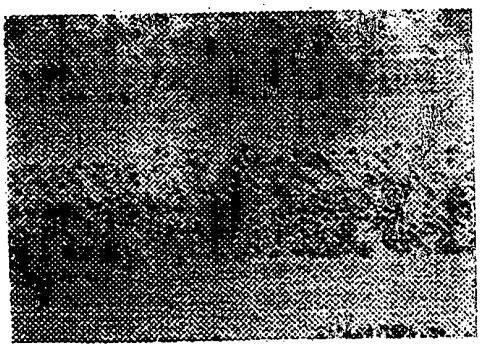

FIG. 2C

FIG. 2D

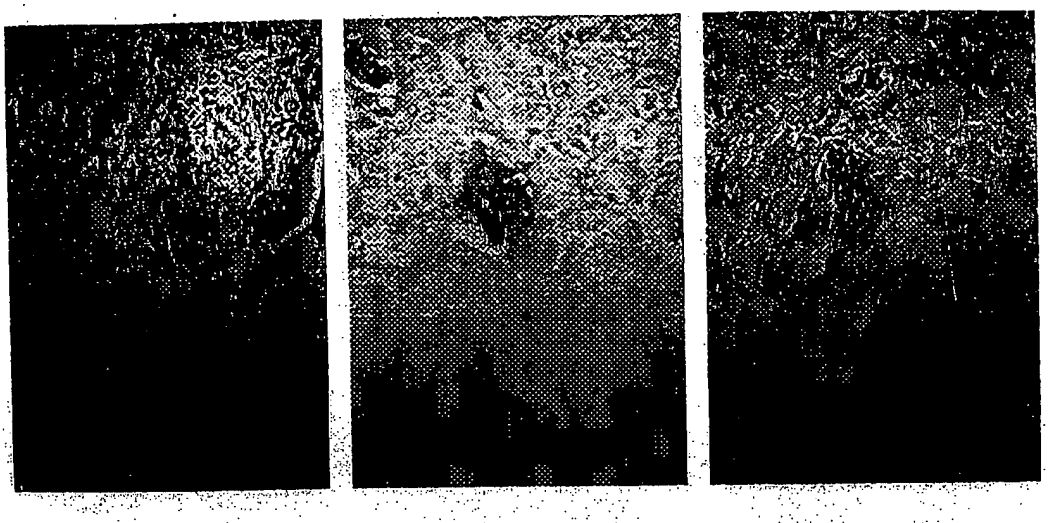

FIG. 3A FIG. 3B FIG. 3C

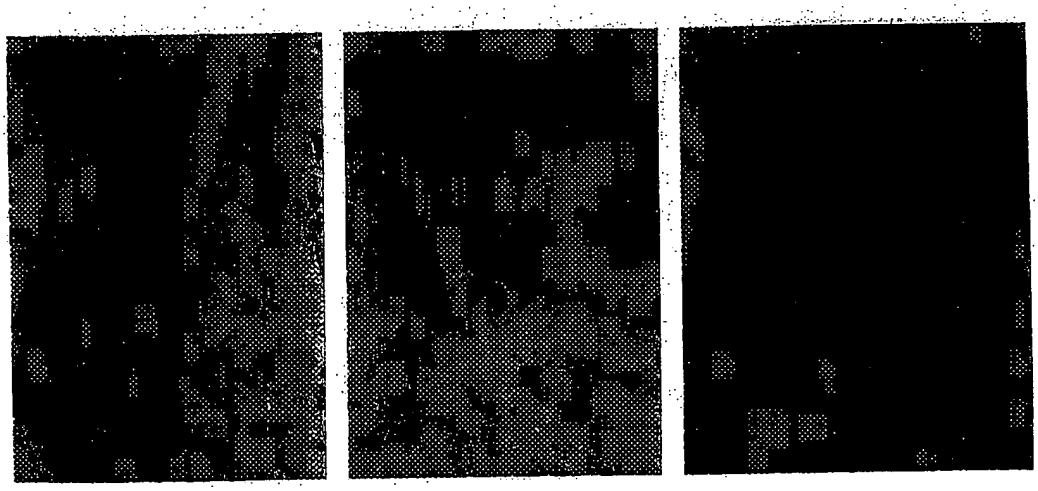

FIG. 4A FIG. 4B FIG. 4C

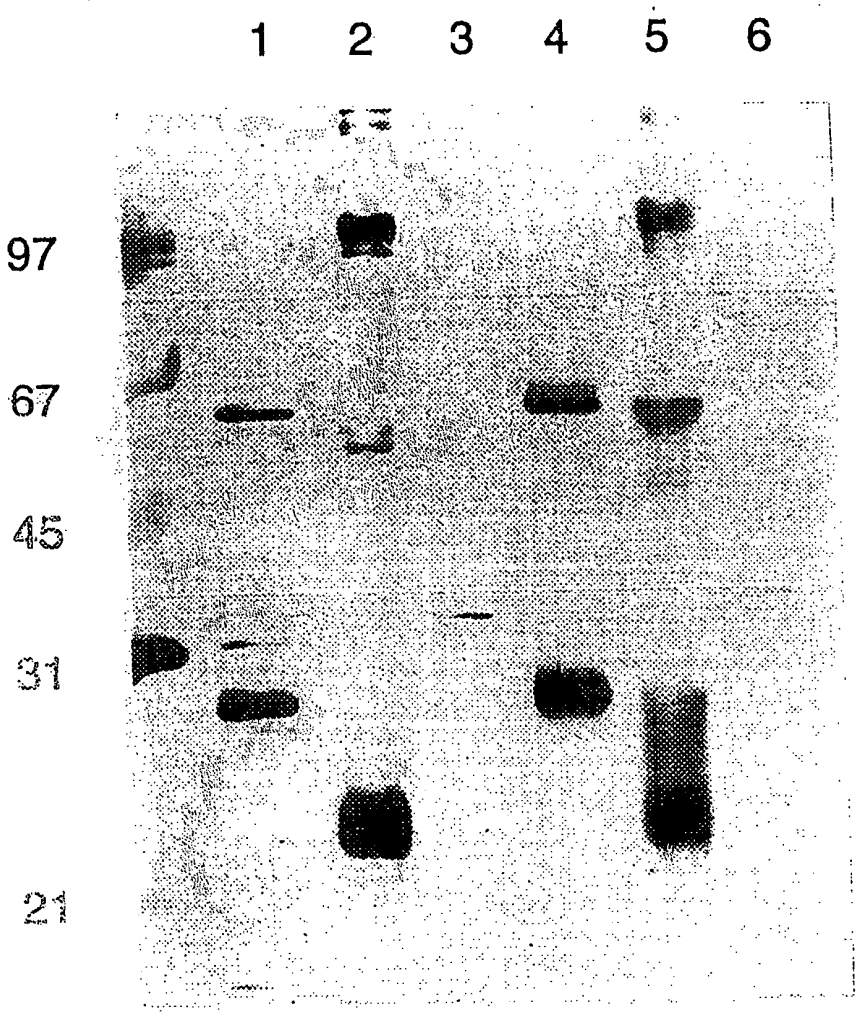

FIG. 5

FIG. 6A

	FR1	CDR1	FR2	CDR2
1.9III	LVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH	WVRQAPGKGLEWVA	VISYDGSNKYYADSVKG
5-3	-EQ---------K----------R	N----	----------	G--S--RK-K-V-----
5-4	-E ---------K----------R	N----	----------	G--S--RK-K-V-----

	FR3	CDR3	FR4
1.9III	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK		
5-3	-------K----------	LSRAGGFDI	WGQGTMVTVSS
5-4	--F----------L----------	DEFSSTRKNFLTGQSKTFAAYYGMDV	WGQGTTVTVSS

FIG. 6B

	FR1	CDR1	FR2	CDR2	FR3
VA27	VLTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDR
5-3	E----------F---------	----RI-T-F--	--------S-----F	D--T--P	-----
5-4	E----------	----G---GS--	-----A---------	---R---	-----

	FR3	CDR3	FR4
VA27	FSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSP	
5-3	--A-W----------	-H----- WT	FGQGTKVEIKR
5-4	-T----------T----------	------QGFT	FGPGTKVDLKR

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/13059

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/53; C07K 16/00
US CL :514/2; 435/7.1, 530/351, 388.7, 388.73
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2; 435/7.1, 530/351, 388.7, 388.73

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, SCISEARCH, DERWENT WORLD PATENT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96/12189 A2 (CEDARS-SINAI MEDICAL CENTER) 25 April 1996, see entire document.	1-20
Y	US 5,292,667 A (PODOLSKY et al.) 08 March 1994, see entire document.	1-20
Y	EGGENA et al. Phage Display Cloning And Characterization Of An Immunogenetic Marker (Perinuclear Anti-Neutrophil Cytoplasmic Antibody) In Ulcerative Colitis. J. Immunology. 1996, Vol. 156, pages 4005-4011, see entire document.	1-20
Y	SEIBOLD et al. Neutrophil Autoantibodies: A Genetic Marker In Primary Sclerosing Cholangitis And Ulcerative Colitis. Gastroenterology. 1994, Vol. 107, No. 2, pages 532-536, see entire document.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
30 OCTOBER 1997

Date of mailing of the international search report
04 DEC 1997

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/13059

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHNABEL et al. Anti-Neutrophil Cytoplasmic Antibodies In Generalized Autoimmune Diseases. Int. Arch. Allergy Immunol. 1996, Vol. 109, pages 201-206, see entire document.	1-20
Y	EGGENA et al. Characterization Of The Ulcerative Colitis Specific pANCA Target Antigen. Gastroenterology. April 1996, Vol. 110, No. 4, page A902, see entire abstract.	1-20